IMPROVEMENTS IN NUTRITIVE VALUE OF CANOLA MEAL WITH PELLETING

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon

By

Xuewei Huang

PERMISSION TO USE STATEMENT

In presenting this thesis in partial fulfilment of the requirements for a Master of Science degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 5A8,
Canada

ABSTRACT

Production of and demand for Canadian canola meal have been increased yearly. In order to improve the competitiveness of canola meal domestically and internationally, as well as to develop potential markets for canola meal, it is necessary to develop canola meal-based products that have high feed values and can be easily transported. The objectives of this research were: 1) to investigate the effects of temperature and time of conditioning during pelleting process on the nutritive values of canola meal in terms of chemical profiles, protein and carbohydrate subfractions, and energy values, using the AOAC procedures, CNCPS v6.1 and NRC (2001), respectively; 2) to detect the effects of temperature and time of conditioning during the pelleting process on rumen degradation and intestinal digestion characteristics and predicted protein supply of canola meal, using the *in situ* procedure, the three-step *in vitro* procedure, and the NRC 2001 model; and 3) to determine pelleting-induced changes in spectral characteristics of molecular structures of canola meal using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) with univariate and multivariate analysis, and reveal the relationship between molecular structures of protein and carbohydrate and nutrient values, rumen degradation and intestinal digestion characteristics, and predicted protein supply of canola meal. Three different conditioning temperatures (70, 80 and 90ºC) and two different conditioning time (50 and 75 sec) were applied in this research. Two different batches of canola meal from a commercial feed company were selected. A randomized complete block design (RCBD) with 3 × 2 factorial arrangement was employed in this research. Molecular spectral functional groups related to protein, cellulosic compounds, and carbohydrates were used in the spectral study. This research indicated: 1) soluble crude protein (SCP) was decreased and neutral detergent insoluble CP (NDICP) was increased with increasing temperature; 2) the lowest protein rumen degradation of pellets was observed at conditioning temperature of 90 ºC and protein rumen degradation was increased by pelleting; 3) the amount of protein digested in the small intestine tended to increase with increasing conditioning temperature; 4) pelleting under different temperatures and time in the current study shifted the protein digestion site to the rumen, rather than to the small intestine; 5) with respect to predicted protein supply, based on the NRC 2001 model, increasing conditioning temperature tended to increase the metabolizable protein supply of canola meal pellets to dairy cattle; 6) changes in the molecular structure of canola meal induced by pelleting can be detected by ATR-FTIR; 7) not only protein molecular structure characteristics but also carbohydrate molecular structure

characteristics play important roles in determining nutrient values, rumen degradation and intestinal digestion characteristics, and the predicted protein supply of canola meal.

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere appreciation to my supervisor Dr. Peiqiang Yu for providing me with this opportunity to be his student. Without his kindness, patience, support, and guidance through the whole program, this work would never have come about. He has inspired me with his positive research ethic and friendly personality. I would also like to express my thanks to my advisory committee—Dr. C. Christensen, Dr. T. Scott and Dr. F. Buchanan—for their patience, encouragement, and valuable suggestions.

I am also thankful to Zhiyuan Niu for his distinguished technical skills. He is such a great help when lab work overwhelmed. Thanks to Louis L. Roth for his engineering experience, for answering my questions, and for giving me suggestions. I would also like to express my thanks to our team members Ling, Yuguang, Ravindra, and Hangshu for sharing their valuable knowledge and contributing to this fulfilling experience.

I would also like to express my thanks to my friends in Canada for their encouragement, company and support, as well as sharing goodwill with me. I wish to convey my appreciation to my family and Qin for their endless support and patience. Without them, I would have never reached the end of my graduate studies.

Last but not least, I would like to acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Saskatchewan Agriculture Development Fund (ADF), the Ministry of Agriculture Strategic Research Chair Program, and SaskCanola.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Lignin
AECP	Truly absorbed endogenous protein in the small intestine
AIA	Amide I area
AIH	Amide I height
AIIA	Amide II area
AIIH	Amide II height
AMCP	Truly absorbed rumen synthesized protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine
ATR	Attenuated total reflectance
BCHO	Rumen bypass CHO
BCP	Rumen bypass CP
BDM	Rumen bypass dry matter
BDNDF	Rumen bypass NDF
CA	Cellulosic compounds area in spectral profiles
CA1	Volatile fatty acids
CA2	Lactic acids with degradation rate of 7%/h
CA3	Organic acids with degradation rate of 5%/h
CA4	Simple sugars with degradation rate of 40-60%/h
CB1	Starch with degradation rate of 20-40%/h
CB2	Soluble fiber with degradation rate of 20-40%/h
CB3	Available NDF with degradation rate of 4-9%/h
CC	Unavailable cell wall
CEL	Cellulosic compounds
CH	Cellulosic compounds height in spectral profiles
CHO	Carbohydrate
CLA	Hierarchical cluster analysis
CNCPS	Cornell net crude protein system
CP	Crude protein
D	Degradable fraction

dBCHO	Intestinal digestibility of bypass CHO
dBNDF	Intestinal digestibility of bypass NDF
DE	Digestible energy
DE_{1x}	Digestible energy at 1x maintenance
dIDP	Digestibility of intestinal digestible protein
DM	Dry matter
DPB^{NRC}	Degraded protein balance in the NRC 2001 model
DVE	Truly absorbed protein in the small intestine in DVE/OEB system
ECP	Rumen endogenous protein
ED	Rumen effectively degradable fraction
ED_N/ED_CHO	Effective degradability ratio of N to CHO
ED_N/ED_OM	Effective degradability ratio of N to OM
EDCHO	Rumen effectively degraded CHO
EDCP	Rumen effectively degraded CP
EDDM	Rumen effectively degraded dry matter
EDNDF	Rumen effectively degraded NDF
EE	Ether extract
ENDP	Endogenous protein loss in DVE/OEB system
FMV	Feed milk value
FTIR	Fourier transform infrared spectroscopy
IDBCHO	Intestinal digestible bypassed CHO
IDBNDF	Intestinal digestible bypassed NDF
IDP	Intestinal digestible protein
Kd	Degradation rate
ME	Metabolizable energy
ME_p	Metabolizable energy at different production levels
MP	Metabolizable protein
N_MCP	Microbial protein synthesized based on available nitrogen
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE	Net energy
NE_g	Net energy for growth
NE_{Lp}	Net energy for lactation at production level

NE_m	Net energy for maintenance
NFC	Non-fiber carbohydrate
NPN	Non-protein nitrogen
OEB	Degraded protein balance in DVE/OEB system
PA1	Ammonia
PA2	Non-ammonia soluble protein
PB1	Moderately degraded protein with degradation rate of 3-20%/h
PB2	Slowly degraded protein with degradation rate of 4-9%/h
PC	Unavailable protein
PCA	Principal component analysis
PDI	Pellet durability index
Ratio1	Ratio of amide I to amide II area
Ratio2	Ratio of α-helix to β-sheet
Ratio3	Ratio of amide I to amide II height
RCBD	randomized complete block design
RDCA4	Ruminally degraded CA4
RDCB2	Ruminally degraded CB2
RDCB3	Ruminally degraded CB3
RDPA2	Ruminally degraded PA2
RDPB1	Ruminally degraded PB1
RDPB2	Ruminally degraded PB2
RU	Rumen undegradable fraction
RUCA4	Ruminally escaped CA4
RUCB3	Ruminally escaped CB3
RUCC	Ruminally escaped CC
RUPA2	Ruminally escaped PA2
RUPB1	Ruminally escaped PB1
RUPB2	Ruminally escaped PB2
RUPC	Ruminally escaped PC
S	Soluble fraction
SCA	SCHO area in spectral profiles
SCHO	Structural CHO
SCP	Soluble crude protein

SCP1	SCHO peak 1 height in spectral profiles
SCP2	SCHO peak 2 height in spectral profiles
SCP3	SCHO peak 3 height in spectral profiles
T0	Lag time
TCA	TCHO area in spectral profiles
TCHO	Total CHO
TCP1A	TCHO peak 1 area in spectral profiles
TCP1H	TCHO peak 1 height in spectral profiles
TCP2A	TCHO peak 2 area in spectral profiles
TCP2H	TCHO peak 2 height in spectral profiles
TCP3A	TCHO 3 area in spectral profiles
TCP3H	TCHO peak 3 height in spectral profiles
TDCHO	Total digestible CHO
tdCP	Truly digestible CP
tdFA	Truly digestible fatty acid
TDN	Total digestible nutrients
TDN_{1x}	Total digestible nutrients at 1x maintenance
TDNDF	Total digestible NDF
tdNFC	Truly digestible NFC
TDP	Total digestible protein
TPSI	Total protein supplied to the small intestine
TRDC	Total ruminally degraded CHO
TRDP	Total ruminally degraded CP
TRUCC	Total ruminally escaped CHO
TRUP	Total ruminally escaped CP
U	Undegradable fraction

1. General Introduction

Canola is the offspring of rapeseed. Its meal portion has low glucosinolate levels and its oil portion has low erucic acid levels. Canola seeds contain 42-43% oil. Canola is the major oilseed crop in Canada (Newkirk, 2009). Canola oil has served as a premium edible vegetable oil with proven benefits for human consumption (Eskin, 2013). Canola meal, a by-product when oil is extracted from canola seeds, has high protein content of 36-39% and good amino acid profiles for animals. Canola protein provides high levels of methionine and cysteine, though its lysine content is limited (Newkirk, 2009). Canola meal is commonly used as an excellent protein source not only for monogastrics but also for ruminants (Newkirk, 2009). Canola meal is considered the second most traded protein supplement around the world, and is traded in pellets or in mash (Newkirk, 2009).

Pelleting, which started more than 80 years ago, is a commonly-used technique in the feed industry (Thomas and van der Poel, 1996). Pelleting is a process that uses moisture, pressure, and heat to produce larger particles by agglomerating smaller particles (Skoch et al., 1981; Falk, 1985). Pelleting improves feed quality. Proven benefits include improving palatability and the hygienic condition of feed, increasing feed bulk density and flowability, solving nutrient segregation problems, and improving transportation efficiency of feed. It also affects the digestion characteristics of feeds. The conditioning stage during pelleting process, which applies heat and moisture to feed, may induce partial protein denaturation and starch gelatinization, and thereby influences protein and starch digestions (Abdollahi et al., 2013). If high temperature applies during feed processing, the Maillard reaction would also happen (Abdollahi et al., 2013). Pellet quality, however, impacts the benefits of pelleting (Thomas and van der Poel, 1996; Thomas et al., 1997, 1998; Abdollahi et al., 2013).

While taking advantage of pelleting in order to improve feed quality, it is necessary to understand the effects of pelleting on nutrient values and the availability of feeds. Canadian canola meal is in great demand, and the Canola Council of Canada also seeks to improve the competitiveness of Canadian canola meal in the feed market. Hence, it is necessary to develop canola meal-based products that have high feed values and can be easily transported. For pelleting, it is vital to understand the effects temperature and time of conditioning have on nutrient values and availability of canola meal. However, conventional chemical analysis cannot assess the inherent structure of the feed because harsh chemical reagents destroy them. In addition, conventional analytical procedures cannot fully explain the changes in nutrient

values and availability of feeds. Hence, assessing the inherent structures of feed ingredients and detecting their changes during feed processing may help researchers understand the effects of feed processing on feed ingredients on a molecular basis (Yu, 2004). The Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) has been used in feed analysis as a rapid, nondestructive, and relatively precise tool to detect the inherent structures of feed ingredients (Xin and Yu, 2013a, b). Hence, the overall objectives of this study were to detect the effects of conditioning temperature and time on chemical profiles, predicted protein supply, inherent structures, protein and carbohydrate fractions, and rumen degradation kinetics and intestinal digestion characteristics of canola meal. Relationships between molecular structures and nutrient values, predicted protein supply, and rumen degradation and intestinal digestion characteristics of canola meal were determined as well.

2. Literature Review

2.1. Development and production of canola

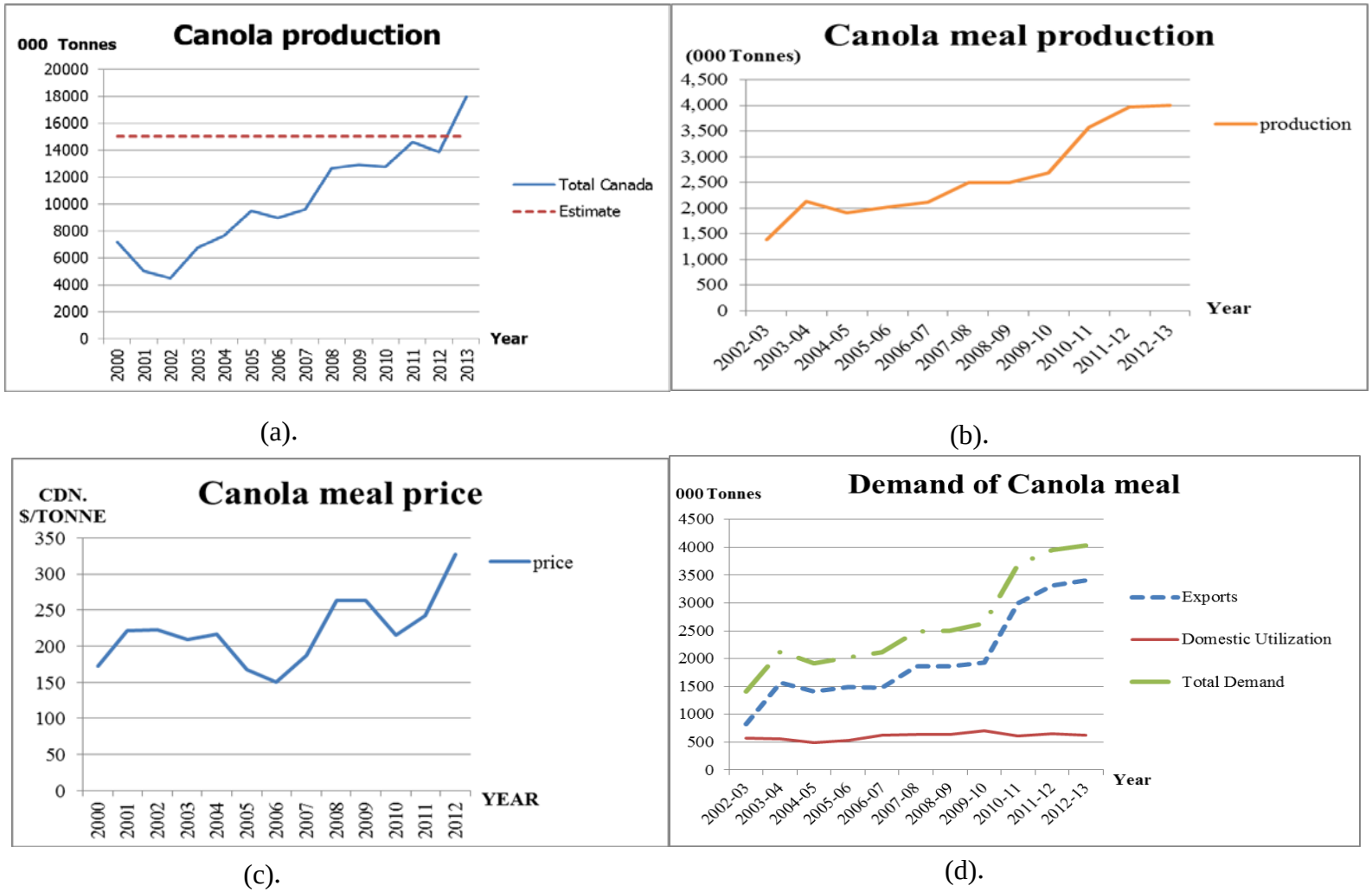
It was documented that rapeseed was first planted in Canada, in Saskatchewan in 1928 (Eskin, 2013). Canola was bred from *Brassica napus* and *Brassica campestris/rapa*, and has low levels of erucic acid (<2%) in the oil portion and low glucosinolates contents (<30 umol/g) in the meal portion (Bell, 1993; Newkirk, 2009; Eskin, 2013). In Canada, *Brassica napus* is the predominant species, accounting for 95% of canola production. *Bassica juncea* and *rapa* (yellow-seeded) and *Brassica napus* (brown-seeded) are the most common types produced in western Canada (Yu, 2007; Newkirk, 2009).

The canola industry contributes $19.3 billion annually to the Canadian economy (Canola Council of Canada, 2013). The canola industry provides 249,000 Canadian jobs and $12.5 billion in wages each year. Canadian wages created by the canola industry today ($12.5 billion in 2012) are more than triple to what they were in the past ($3.4 billion in 2004) (Canola Council of Canada, 2013). Figure 2.1 (a) shows that the production of canola exceeded 15 million tonnes in 2013. Figure 2.1 (b) shows that the rate of canola meal production rate continually increased, and almost reached 4 million tonnes in 2013. Figure 2.1 (d) shows that demand for canola meal for export continued to increase in the last decade.

2.2. Features of canola

2.2.1. Features of canola seed

The canola seed is small, oblong, spherical, or slightly laterally flattened, has a highly lignified coat and a diameter ranging between 1.5 and 2.5 mm (Barthet and Daun, 2011). Canola is either yellow-seeded or brown-seeded (Heendeniya, 2008; Barthet and Daun, 2011). Canola seeds contain around 14% moisture, 42 to 43% oil, and 20% crude protein (CP) (Heendeniya, 2008, Newkirk, 2009; Barthet and Daun, 2011). The carbohydrate components in canola are divided into three subfractions: soluble sugars, insoluble carbohydrates, and fibers (Barthet and Daun, 2011). The seed coat colour is controlled by the gene in the *Brassica* species. Yellow seed colour is recessive to dark seed colour (Rashid et al., 1994).



Source: Canola Council of Canada (2013)

Figure 2.1 Production and demands of canola and its products: (a). Production of canola. (b). Production of canola meal; (c). Price of canola meal; (d). Demand of canola meal

Compared with brown-seeded canola, yellow-seeded canola has a thinner palisade layer, and, therefore, a lower proportion of polyphenol and lignin (Rahman et al., 2001). Further, yellow-seeded canola meal is higher in sucrose, protein, and oil, similar in oligosaccharides, and lower in fiber (Simbaya et al., 1995).

2.2.2. Canola processing and canola meal

2.2.2.1. Canola processing

Canola oil is usually extracted from canola seeds and separated from meal using pre-press solvent extraction (Newkirk, 2009). Selecting mature and good quality canola seeds is an important step for obtaining high quality products (Unger, 2011).

Canola seeds are cleaned at crush plants to remove the dockage materials. Canola seeds are normally heated to 35 to 45ºC for 35 to 45 min before flaking, in order to avoid shattering in the flaking process. This is especially required for crush plants in cold climates (Newkirk, 2009; Unger, 2011).

The resulting flakes will be cooked or conditioned at 80 to 105ºC for 15 to 20 min; the optimal temperature is 88ºC (Newkirk, 2009). Cooking thermally ruptures oil cells, improves oil droplet coalescence, adjusts moisture content, inactivates hydrolytic enzymes, and reduces flake oil viscosity (Newkirk, 2009; Unger, 2011). A cooking temperature up to 120ºC can be used when rapeseeds have high glucosinolates levels (Newkirk, 2009). A stacked cooker or horizontal rotary conditioner is usually used (Unger, 2011).

Screw presses or expellers are usually used to remove as much oil as possible from the cooked canola flakes; then, canola presscake is produced (Newkirk, 2009). During this process, usually 50 to 70% of the oil is pressed out, and the volume is reduced by 13 to 14 times (Newkirk, 2009; Unger, 2011).

Canola presscake contains around 18 to 20% oil (Newkirk, 2009). The remaining oil is typically removed via solvent extraction, with hexane. Before solvent extraction, the presscake is usually cooled from 100ºC to around 60ºC to prevent the evaporation of hexane. When presscake enters the extractor, miscella (oil and solvent) or solvent will flood the extractor. After washing with pure solvent, the solvent-extracted meal is hexane-saturated and called “marc”; its oil content ranges between 0.6 and 0.9% (Newkirk, 2009; Unger, 2011).

The marc is discharged from the extractor and then deposited in a desolventizer toaster vessel. During desolventizing-toasting, the temperature is carefully controlled to heat the marc to 95 to 115ºC for 30 min. Meal solvent content decreases from 25 to 35% to less than 250 parts per million (ppm) after desolventizing-toasting (Newkirk, 2009).

2.2.2.2. Canola meal

The protein content of Canadian canola meal is guaranteed at 36% (8% moisture), while the actual protein content is around 36-39% (Newkirk, 2009). Canola meal is considered a premium protein source for animal feeding (Newkirk et al., 2003; Newkirk, 2009). Canadian canola meal has a higher oil content than that from other countries (ca. 3.5 vs. 1-2%) because of the process of adding "gums" back into the meal during the desolventizing-toasting process (Newkirk et al., 2003; Newkirk, 2009).

Canola meal has very complex carbohydrate matrices (Newkirk, 2009). The fiber content of canola meal is around 12% (Bell, 1993; Newkirk, 2009). The canola hull, which accounts for up to 30% of the meal, contributes to this (Bell, 1993; Newkirk, 2009). Dockage materials entering the meal at the end of processing also increase its fiber content (Heendeniya, 2008).

2.2.3. Characteristics of canola protein

There are three major protein classes in canola seeds: albumins, globulins, and oleosins (Barthet and Daun, 2011). Albumins are water soluble; globulins are salt soluble. The metabolism of canola seed is controlled by specific enzymes in albumins. The major storage protein is contributed by globulins, which make up to 70% of the seed protein (Barthet and Daun, 2011). Storage proteins in canola seeds are divided into two types: napin and cruciferin. The water-soluble, small, and basic napins (1.7S globulin) make up to 20% of the seed protein. Cruciferins (12S globulin) make up to 50% of the seed protein (Barthet and Daun, 2011). The oleosins, important for their oil-storing and structural functions, account for around 20% of the seed protein (Huang, 1992; Murphy, 1993; Barthet and Daun, 2011). Oleosins are water insoluble and can be isolated using flotation with hexane (Murphy, 1993).

2.3. Utilization and benefits of inclusion of canola meal in animal feeds

As a protein supplement, a critical factor for canola meal in diets is its available energy (Bell, 1993). Because of its relatively low lysine and limited energy levels, canola meal has a higher feed value for animals that require intermediate levels of lysine and energy and high levels of methionine and cysteine. Nevertheless, for animals that require high levels of lysine and energy, canola meal would have a lower feed value (Arntfield and Hickling, 2011). Canola meal is widely used in diets for poultry, cattle, and swine (Newkirk, 2011).

2.3.1. Utilization and benefits of canola meal in diets for non-ruminants

Canola meal can be used in all types of poultry diets (Newkirk, 2009). Layers can maintain a high level of egg production when they consume diets containing canola meal (Newkirk, 2009). Feeding canola meal to layers based on the digestible amino acid content did not affect the layers' performance or egg weights (Novak et al., 2004; Newkirk, 2009). Because of the concern of liver-hemorrhage mortality, a level of 10% canola meal in layers' diets is usually set (Newkirk, 2009). Thirty percent of the diet for broiler chickens based on digestible amino acid content can be substituted using canola meal without impairing animal performance (Newkirk, 2009; Arntfield and Hickling, 2011).

Canola meal is widely accepted as an economical, effective feed ingredient for swine (Newkirk, 2009). However, its high fiber content and relatively low available energy limit its benefits for swine. Its inclusion rate in diets may vary depending on the interaction of price and nutrient values (Newkirk, 2009). The diet for starters can contain up to 5% canola meal to help them adapt subsequent diets containing higher levels of canola meal. Up to 25% of the diet for growing and finishing pigs can be substituted with canola meal without impairing animal performance (Newkirk, 2009). Canola meal also can be used as the main protein source for breeding swine in all reproductive phases (Newkirk, 2009).

2.3.2. Utilization and benefits of canola meal in ruminant rations

Canola meal is an excellent protein source for both dairy and beef cattle (Newkirk, 2009). Spörndly and Åsberg (2006) observed that canola meal has good palatability, which improves feed intake. In addition, canola meal exhibits relatively higher *in situ* rumen degradability than other protein supplements such as corn gluten, fish, and blood meals (Piepenbrink and Schingoethe, 1998; Harstad and Prestløkken, 2001; Newkirk, 2009). Canola

meal with high rumen degradability supplies sufficient ammonia, peptides, and amino acids, not only for microbial growth and microbial protein synthesis in the rumen but also for growth and lactation in dairy cattle (Boila and Ingalls, 1992; Newkirk, 2009). Brito and Broderick (2007) observed that canola meal supports higher milk fat and protein content than soybean or cottonseed meals. Cows fed canola meal had 1.0 kg more milk production than those fed cottonseed or soybean meal (Newkirk, 2009). Schingoethe (1991) reported that canola meal has a higher milk protein score than other protein supplements such as corn gluten meal, soybean meal, sunflower meal, and corn dried distillers grains with solubles (DDGS). Canola meal is also acceptable for beef cattle. The maximum level of canola meal allowable in finishing diets is 10%. Up to 15% of the dry matter of the diet for backgrounding cattle can be substituted with canola meal (McKinnon et al., 2014).

2.4. Benefits of pelleting for the animal feed industry

Feed is a large portion of the cost of animal production. Feed processing may further increase the feed cost, and thus increasing the total cost of animal production (Nolan et al., 2010). However, feed processing provides opportunities to add value to feedstuffs and consequently improve animal performance (Abdollahi et al., 2013).

2.4.1. Introduction of pelleting

Pelleting is a common feed processing technology, and its purpose is to agglomerate smaller particles into larger particles using moisture, heat, and pressure (Skoch et al., 1981; Falk 1985). Generally, grinding and mixing, conditioning, pelleting, and cooling are the main procedures of the pelleting process (Thomas and van der Poel, 1996; Thomas et al., 1997). First, larger feed particles will be ground into smaller ones, and then mixed into diet. Then, the mixed ground mash is passed to the conditioner. During the conditioning stage, feed mash gains an enhanced binding ability using water, heat, and pressure (Skoch et al., 1981). Steam is commonly used during the conditioning stage to increase the moisture content and temperature of the feed materials (Thomas and van der Poel, 1996; Abdollahi et al., 2013). The conditioning process can improve hygienic conditions of feed materials by inhibiting microbial bioactivity (Thomas and van der Poel, 1996). Conditioning with heat and moisture may also soften feed, denature protein, and gelatinize starch (Kinsella, 1979; Skoch et al., 1981, 1983; Rao and Lund, 1986). The single-pass conditioner is commonly used, but there are other types of conditioners available, such as the double- or triple-pass conditioners, the

jacketed conditioner and the pressure conditioner (Fairfield et al., 2005). Feed mash is usually conditioned at 65 to 90ºC, and then is passed to the pelleting chamber and pressed through a die to form pellets (Yu et al., 2002). After leaving the pellet die, pellets have a moisture level of 12 to 17.5% and a temperature of 60 to 95ºC (Thomas et al., 1997; Abdollahi et al., 2013). The cooling stage decreases the moisture content and temperature of the pellets for safe storage (Maier and Bakker-Arkema, 1992; Thomas et al., 1997). A moisture level of 10 to 12% is desirable (Abdollahi et al., 2013). Horizontal crossflow coolers and counterflow bunker coolers are commonly used (Thomas et al., 1997).

2.4.2. Physical quality of pellets

The physical quality of pellets describes the ability pellets have to withstand attrition stress during manufacturing and handling in the factory and feeding activities in the farm without breaking up and generating fines (Thomas and van der Poel, 1996; Cramer et al; 2003; Amerah et al., 2007). Pellet durability and pellet hardness are two parameters used for evaluating the physical quality of pellets (Thomas and van der Poel, 1996). The pellet durability index (PDI) is usually used (ASAE, 1997). Pellet durability can be measured according to the "Pfost" procedure (Thomas and van der poel, 1996). Five hundred grams of sieved pellets were put in a drum with specified dimensions and then tumbled at 50 rpm for 10 min. After tumbling, pellets were sieved again. Pellets that did not pass through the sieve were measured. The ratio of the weight of pellets after tumbling to the weight of pellets before tumbling is calculated as the PDI. Pellet hardness is another measurement of pellet quality and can be defined as the necessary force to crush a pellet. The "Kahl" device is usually used to measure pellet hardness (Abdollahi et al., 2013).

2.4.3. Advantages of pelleting for the feed industry and animal nutrition

Pellets have a better flow property than mash, which is good for transporting on conveying belts and discharging from silos (Thomas and van der Poel, 1996). Pellets have higher bulk density and transportation efficiency than mash feeds. More product can be shipped in each load (Thomas and van der Poel, 1996). The pellet ingredients are well blended and mixed and will thereby stay in the pellet form. This solves segregation problems. Also, pelleting improves the hygienic condition of feed by reducing microbial bioactivity (Thomas and van der Poel, 1996; Abdollahi et al., 2013). Behnke (1994) indicated that pelleting can increase feed palatability, which encourages animals' appetite and enhances

feed intake. In addition, pelleting can reduce feed wastage by preventing selective consuming and sorting (Abdollahi et al., 2013). Pelleting can reduce the dustiness of feed, which is associated with respiratory system health of humans and animals. Pelleting can inactivate heat-labile anti-nutritional factors, and may change nutrient digestion characteristics. Starch can be partially gelatinized during the pelleting process and its availability for enzymatic degradation may be improved (Abdollahi et al., 2013).

Pelleting may denature protein and cause Maillard reactions (Abdollahi et al., 2013). The products of Maillard reactions are considered unusable for animals; whereas, denaturation may improve protein digestion by exposing new sites for enzymatic attack (Camire et al., 1990; Voragen et al., 1995; Abdollahi et al., 2013). However, the effects of pelleting on protein digestibility remain inconsistent in literature due to the different processing methods and conditions (Abdollahi et al., 2013).

2.5. Feed evaluation

2.5.1. Application of Cornell Net Carbohydrate and Protein System (CNCPS) v6.1 in feed evaluation

The Cornell Net Carbohydrate and Protein System (CNCPS), which was designed as a mathematical model, incorporates basic principles of rumen function, animal physiology, and feed digestion and passage to evaluate animal performance and rations for all classes of cattle in all production situations (Fox et al., 2004; Tylutki et al., 2008). The CNCPS provides accurate evaluations of feed intake, nutrient requirements, body reserves, rumen degradation and intestinal digestion of nutrients, and nutrient extraction (Fox et al., 2004; Tyutki et al., 2008). The CNCPS has proven advantageous in predicting metabolizable energy (ME), rumen N and amino acids availability (Lanzas et al., 2008).

In CNCPS v6.1, protein pools are divided into three main fractions based on their ruminal degradation characteristics: PA (ammonia and soluble non-ammonia CP), PB (true protein) and PC (unavailable protein). PA is further divided into two subfractions: PA1 (ammonia) and PA2 (soluble non-ammonia protein). The PA1 subfraction instantly degrades at 200%/h. The PA2 subfraction degrades at 10 to 40%/h. The PB is divided into two subfractions: PB1 and PB2. The PB1 subfraction is the moderately degraded protein containing neutral detergent soluble protein, and degrades at 3 to 20%/h. The PB2 subfraction contains proteins which are bound in fiber, and slowly degrades at 4 to 9%/h in forages. The

PC is insoluble in acid detergent and unavailable for ruminants (Tylutki et al., 2008; Van Amburgh et al., 2010; Higgs et al., 2012; Van Amburgh et al., 2013).

Carbohydrate pools contain eight subfractions: CA1, CA2, CA3, CA4, CB1, CB2, CB3, and CC (Van Amburgh et al., 2010). The CA1 subfraction includes the volatile fatty acids (VFAs), such as acetate, propionate, and butyrate. The CA2 subfraction is lactic acid which degrades at 7%/h. The CA3 subfraction, which contains organic acids such as citric, malic, and aconitic acids, degrades at 5%/h. The CA4 subfraction is simple sugars and degrades at 40 to 60%/h. The CB1 subfraction is starch and degrades at 20 to 40%/h. The CB2 subfraction contains the soluble fibers, which include pectic polysaccharides, β-glucans and fructans, and degrades at 20 to 40%/h. The CB3 subfraction is available NDF and degrades at 4 to 9%/h. The CC, which consists of resistant starch and unavailable cell walls with lignin, is unavailable for animals (Tylutki et al., 2008; Van Amburgh et al., 2010; Higgs et al., 2012; Van Amburgh et al., 2013).

2.5.2. Energy estimation in feed ingredients

In order to optimize animal production with desirable quality, formulate appropriate rations, maintain animal health and behavior, and control feed cost with minimal wastage of feed ingredients, the precise evaluation of energy levels of feed ingredients is required (Weiss, 1998). The terms used to express available energy in feeds include total digestible nutrient (TDN), metabolizable energy (ME), digestible energy (DE), and net energy (NE). The TDN can be determined using summative approaches described in NRC (2001) for dairy cattle and NRC (1996) for beef cattle. In NRC (2001), truly digestible non-fiber carbohydrate (tdNFC), truly digestible crude protein (tdCP), truly digestible fatty acid (tdFA), and truly digestible neutral detergent fiber (tdNDF) are used to determine TDN at 1x maintenance (TDN_{1x}). These parameters can be calculated based on basic chemical profiles of feed (Weiss et al., 1992; NRC, 2001).

Tyrrell and Moe (1975) indicated the diet digestibility decreases when feed intake increases. This is because the time feed stays in digestive system would be decreased when feed intake increases (NRC, 2001). Therefore, the energy values of diets fed to animals was decreased by increasing intake, which is very important for dairy cows at a high production level that can reach up to four times the intake to supply maintenance (NRC, 2001).

The “discount factor” concept has been incorporated to obtain adjusted energy values at different levels of feed intake. The discount factor is further applied when calculating digestible energy at a production level (DE_p), ME at a production level (ME_p), net energy for lactation (NE_L), net energy for maintenance (NE_m) and net energy for gain (NE_g) (NRC, 1996, 2001).

2.5.3. Assessing rumen degradation kinetics of feed ingredients using the *in situ* technique

To provide sufficient nutrients to ruminants and optimize their performance and production, it is necessary to understand feed degradation characteristics in the rumen. The first attempt to understand feed degradation characteristics in rumen can be traced back to the 1930s (Ørskov et al., 1980). Although more precise and reliable laboratory techniques have been developed, the *in situ* nylon bag technique with the simple procedure of weighing sample is still famous and widely used to determine rumen degradation kinetics in feed materials (Ørskov et al., 1980). Not only fast, this technique can also handle many samples at once (Nocek, 1988). Therefore, this technique is a powerful tool to evaluate feedstuffs, and assist researchers in understanding how feed degrades in the functioning rumen (Ørskov et al., 1980). Both nonlinear and logarithmic-linear transformation models are used to estimate degradation parameters in the nylon bag technique. The first order kinetics model was developed by Ørskov and McDonald (1979), and has been used extensively. The model accuracy was improved by Robinson et al. (1986) and Dhanoa (1988) by incorporating the “lag time (T0)” concept.

Feed proteins need to break down into non-protein nitrogen (NPN) before being incorporated in microbial protein synthesis. Further, energy generated from the digestion of carbohydrate is another essential factor for microbial protein synthesis. Hence, the balance between N and energy supply is important for optimizing microbial protein production (Tamminga et al., 1994; NRC, 2001; Liu et al., 2012). Sinclair et al. (1993) outlined the formula to calculate the hourly effective degraded content as follows:

$$\text{Hourly ED (g/kg DM)} = S + [(D \times K_d) / (K_p + K_d)] \times [1 - e^{-t \times (Kd + Kp)}],$$

where, D (%) is potentially degradable fraction of nutrients and K_p (%/h) is the passage rate of nutrients outflow from rumen. The optimal assumed ratios are 25 g N/kg OM and 32 g N/kg CHO degraded in the rumen (Czerkawski, 1986; Tamminga et al., 1990; Sinclair et al., 1991, 1993).

The quantity of nutrient degraded per hour is obtained by differencing the cumulative amounts between successive hours (Nuez-Ortín and Yu, 2010). Calculations for Hourly ED ratio of CHO to N or OM to N are detailed in literature (Nuez-Ortín and Yu, 2010; Yang et al., 2013a).

2.5.4. Three-step *in vitro* procedure for estimating intestinal digestibility of feed nutrients

Microbial protein and dietary N passed from the rumen to the duodenum determine the amount of total absorbable protein (NRC, 2001). Increasing rumen undegraded protein that is digestible in the small intestine becomes much more important (Calsamiglia et al., 1995). Calsamiglia et al. (1995) developed a three-step *in vitro* procedure, which has been adopted by NRC (2001), as a reference method (Gargallo et al., 2006). The combination of the *in situ* technique and the three-step *in vitro* procedure provided clear insights into protein digestion in ruminants (Calsamiglia et al., 1995). Fifteen grams of samples, which from residuals after rumen incubation of 12 or 16 h, are incubated in 10 mL 0.1 mol/L HCl solution containing pepsin at 38 ºC for 1 h in a shaker water bath and then neutralized using 0.5 mL 1 mol/L NaOH and 13.5 mL phosphate buffer (pH = 7.8) containing pancreatin. Then the mixture is incubated for 24 h at 38 ºC in a shaker water bath. During incubation, samples need to vortex about every 8 h. After incubation, 3 mL of 100% Trichloroacetic acid (TCA) solution is added into the mixture in order to stop enzymatic activity and make undigested protein precipitate. After then, samples are centrifuged and analyzed supernatant for soluble N using the Kjeldahl method (AOAC, 984.13). The ratio of TCA-soluble N to N in the sample expressed in percentage basis is considered as the digestibility of protein in the small intestine (Calsamiglia and Stern, 1995; NRC, 2001; Gargallo et al., 2006).

2.5.5. Prediction of truly digestible protein supply to small intestine

Adequate absorbed true protein can supply sufficient quantities of amino acids for dairy cows, which ensures high milk production. Dairy cows not only need sufficient available N for microbial protein synthesis, but also require enough protein bypassed from the rumen and absorbed in the small intestine (Yu, 2005a).

2.5.5.1. The NRC 2001 dairy model

The NRC 2001 model predicts the truly digested and absorbed protein in the small intestine and the degraded protein balance. Metabolizable protein (MP), which is defined as the protein digested and absorbed in the form of amino acids in the small intestine, is incorporated into the NRC 2001 model. The NRC 2001 model is based on the TDN value, and the endogenous protein passed to the small intestine is considered a contribution to the total MP. According to NRC (2001) and Yu (2005a), MP is calculated as the sum of truly absorbed rumen undegraded protein in the small intestine, truly absorbed rumen synthesized protein in the small intestine, and the truly absorbed endogenous protein passed to the small intestine. The concept of degraded protein balance in the NRC 2001 model is derived from the DVE/OEB system, and calculated as rumen degraded protein minus the product of 1.18 and microbial protein synthesized based on the available energy for rumen fermentation.

2.6. Application of Mid-IR spectroscopy in feed science

2.6.1. Infrared spectroscopy

Infrared spectroscopy measures IR light frequencies absorbed by samples. The application of infrared spectroscopy enables the detection of molecular structures of either known or unknown samples because of the correlation between some molecular structures and peak positions in the infrared spectrum. In addition, infrared spectroscopy can determine the difference between samples by comparing spectra of samples and investigating peak positions, heights, and widths.

Different kinds of electromagnetic radiation can be classified based on different wavenumbers (Smith, 2011). Infrared radiation (IR), which is related to molecular vibration, is defined as the electromagnetic radiation with frequencies between 14000 and 4 cm^{-1}. The infrared region is further divided into three subregions: near-IR (14000 to 4000 cm^{-1}), mid-IR (4000 to 400 cm^{-1}) and far-IR (400 to 4 cm^{-1}). The mid-IR region is usually used in mid-infrared spectroscopy (Smith, 2011). Elmore et al. (2005) lists the functional groups of agri-food materials used in infrared imaging.

Fourier Transform Infrared (FTIR) spectroscopy is based on the principles of interferometry and the mathematical Fourier-transformation method (Stuart, 2004). An interferometer is the core of every FTIR machine. The Michelson interferometer is the

common one (Smith, 2011). There are four arms in Michelson interferometers. The top arm consists of the infrared source and a collimating mirror. The bottom arm contains a fixed mirror. The left arm has a moving mirror. Samples and the detector are placed at the right arm. A beamsplitter is located in the heart of the interferometer. The IR beam from the infrared source goes to the collimating mirror to produce parallel rays towards the beamsplitter. Then the beam is divided by the beamsplitter: one (50%) travels to the fixed mirror, the other (50%) moves towards the moving mirror. The beams reflected by the fixed and moving mirrors will meet at the beamsplitter and recombine to form a final beam. The combined beam leaves the interferometer and passes through samples, and then is received by the detector to generate an interferogram (Smith, 2011). The interferogram is a plot of the light intensity versus the optical path difference, and is processed using the Fourier-transformation method (Stuart, 2004; Smith, 2011). The frequencies making up a signal can be analyzed by using Fourier-transformation method (Smith, 2011).

Compared with other types of infrared instruments, FTIR can provide easy, fast and precise measurements with good signal-to-noise ratios (SNRs), allows high throughout and multiplex scans. As well, FTIR requires little sample preparation, and is labor-saving.

2.6.2. The Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Attenuated total reflectance (ATR) FTIR is based on internal reflectance. A high refractive crystal is placed close to the margin of the samples with a low refractive index, and then a beam of light from the radiation source travels through the crystal (Smith, 2011). The angle between the refracted beam and surface normal is called the angle of refraction (Smith, 2011). When the angle of refraction becomes 90^{o}, total internal reflectance occurs. This means the incoming beam will not be reflected or refracted (Smith, 2011). The minimum angle of incidence required to cause total internal reflectance is called the critical angle (Stuart, 2004; Smith, 2011). On ATR-FTIR, when a sample contacts an evanescent wave, which is part of beam wavelength that penetrates beyond the reflecting surface, part of light will be absorbed by the samples, but the rest of the light will be caught by the detector (Stuart, 2004; Smith, 2011). Therefore, ATR is used to describe the attenuated intensity of the total reflected infrared beam after the beam absorbed by the samples (Smith, 2011). ATR-FTIR is non-destructive and cost-effective, requires little sample preparation, and can handle multiple kinds of samples (Smith, 2011).

2.6.3. Methods for spectral analysis

Understanding information in spectra related to biological meaning helps researchers assess not only chemical but also biological information of materials. Univariate and multivariate analyses are two commonly used methods (Yu, 2005b, c, d).

2.6.3.1. Univariate analysis

In the univariate approach, specific observations in spectra such as band frequencies, integrated intensities, band height and area intensities are extracted by researchers to construct maps of spectral data on a mathematical basis (Yu, 2006). The spectral information is connected with biological meaning using mathematical methods (Yu, 2005b).

2.6.3.2. Multivariate analysis

The accuracy of location and concentration of functional groups predicted by the univariate approach cannot be 100% guaranteed (Yu, 2005b, d). It is difficult for researchers to handle large amounts of spectral data with the univariate approach and extract all useful information in datasets. Hence, chemometric methods are preferred (Yu, 2005b, d). Hierarchical cluster analysis (CLA) and principal component analysis (PCA) are the two most commonly used multivariate methods, and require no prior knowledge of spectroscopy (Martin et al., 2004; Yu, 2005b, d). The CLA is based on similarities between spectra, and its results are presented as dendrograms (Yu, 2005b, d, 2006). At first, the distance matrix, which reveals similarities of spectra, will be calculated. Then two similar spectra with a minimal distance matrix will be picked and then formed a new objective called a “cluster”. The spectral distance among remaining spectra will be recalculated and resorted to generate new clusters using the algorithm process. These processes are performed over and over again. Finally, a visible tree diagram will be obtained (Yu, 2005 b, d).

The PCA is based on statistical data reduction. This method transforms an original dataset with interrelated variables into a new dataset that maintains as much information from original dataset as possible. The new dataset consists of uncorrelated variables called principal components (PCs), and more than 95% of the variance in the new dataset can be taken by the first few independent PCs (Martin et al., 2004).

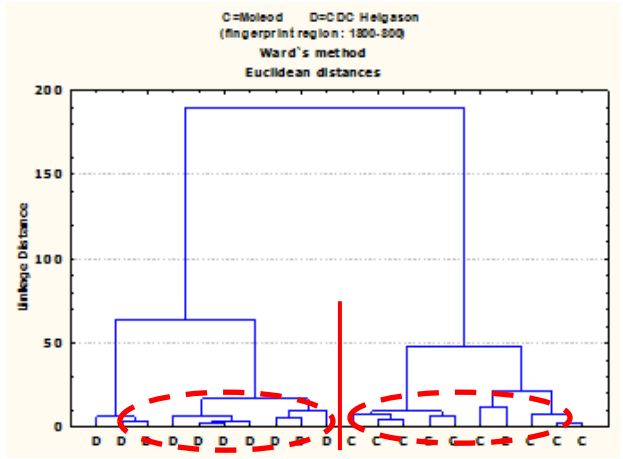
Figure 2.2 shows examples of PCA and CLA from Liu (2009). The separate class in CLA means the cluster only contains similar spectra. Figure 4.4 (a) demonstrated that two separate classes were distinguished in CLA when comparing two different barley varieties of and McLeod at the fingerprint region (ca. 1800 to 800 cm^{-1}). Therefore, CDC Helgson and McLeod could be discriminated with CLA multivariate analysis. Nevertheless, no separate class was observed in CLA when comparing CDC Helgson with another barley variety of CDC Trey (Figure 4.4 (c)). All clusters contained combinations of spectra from the other groups, indicating CDC Helgson and CDC Trey showed similarities of spectra data in the fingerprint region. Figure 4.4 (b) showed comparison of CDC Helgson to McLeod using PCA. Two separate ellipses were grouped, indicating CDC Helgson was different from McLeod. This supported the result from CLA in Figure 4.4 (a). Figure 4.4 (d) showed comparison of CDC Helgson to CDC Trey using PCA. Two ellipses were heavily overlapped and not grouped separately; indicating CDC Helgson was similar to CDC Trey in spectra characteristics at fingerprint. This was supported by CLA (Figure 4.4 (c)).

2.6.4. Application of ATR-FTIR in feed analysis

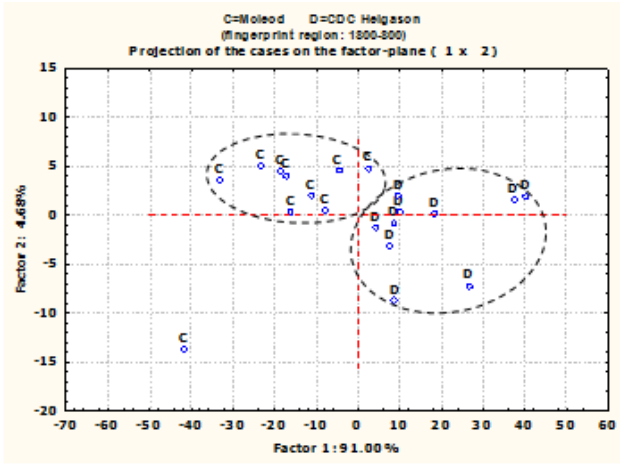
The ATR-FTIR has been applied in fields of physics, chemistry, biology, environmental science and agriculture. In feed analysis, along with univariate and multivariate analysis, ATR-FTIR has helped reveal, on a molecular basis, nutrient quality and utilization of animal feeds (Xin and Yu, 2013a, b; Yang et al., 2013b, 2014). For example, ATR-FTIR has been used to detect the differences between feed materials, investigate effects of gene modification on spectral characteristics of feed ingredients, determine effects of feed processing on chemical and structural information of feed ingredients, and evaluate the effects of rumen degradation on protein and carbohydrate structures of feed (Abeysekara et al., 2012, 2013; Gamage et al., 2012; Jonker et al., 2012; Zhang and Yu, 2012a, b; Yang et al., 2013b, 2014; Peng et al., 2014a; Samadi et al., 2013; Theodoridou and Yu, 2013a; Xin and Yu, 2013 a, b; Zhang et al., 2014).

2.7. Literature summary, conclusion, research objectives, and hypothesis

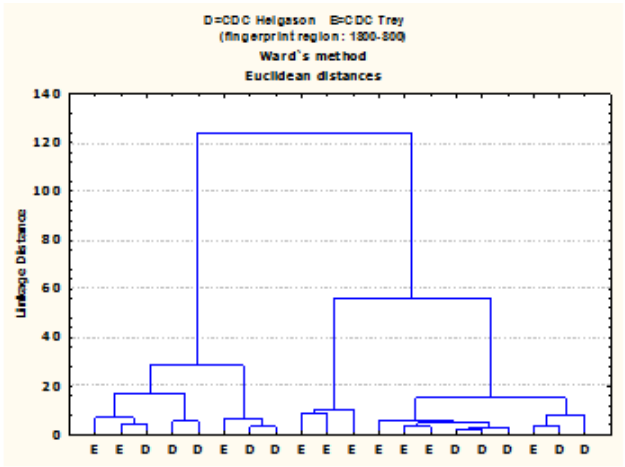
It is possible that certain feed processing techniques can ameliorate animal performance and/or improving feed value (Nolan et al., 2010). Therefore, it is worth investigating the effects of temperature and time of conditioning during the pelleting process on nutrient values and availability of canola meal.



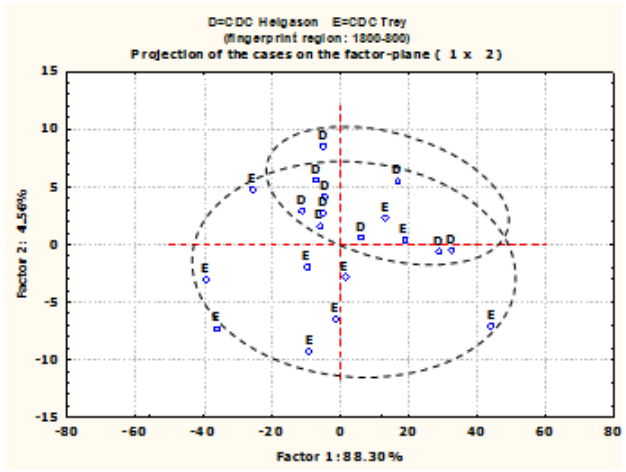
(a).



(b).



(c).



(d).

Adapted from: Liu (2009)

Figure 2.2 CLA and PCA analysis of different barley varieties: comparison of CDC Helgson (D) with McLeod (C) and CDC Trey (E) at fingerprint region of ca. 1800 to 800 cm^{-1}. (a). Comparison of CDC Helgson and McLeod using CLA; (b). Comparison of CDC Helgson and McLeod using PCA; (c). Comparison of CDC Helgson and CDC Trey using CLA; (d). Comparison of CDC Helgson and CDC Trey using PCA. In PCA, scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2) were presented (adapted from Liu (2009)).

Developing an international market for canola meal industry is the key to maintaining and increasing business and maximizing profits for producers. Suitable canola meal-based products should have high feed values and be easily transportable, with improved or undamaged nutrient quality. In Tianjing, China, large amounts of soybean meal and alfalfa are imported from the U.S.A for dairy cattle, while canola meal from Canada is seldom used. Hence, the potential global market opportunities are large.

There is limited information about how temperature and time of conditioning during the pelleting process on the nutrient values of canola meal, or about how pelleting changes its inherent molecular structure. Therefore, there is a need to systematically investigate the differences in nutrient values, protein molecular structures and the availability of canola meal in relation to nutrient utilization in dairy cattle when different conditioning temperatures and time are used during the pelleting process.

2.7.1. Objectives

The objectives of this study were:

- To determine the effects of temperature and time of conditioning during the pelleting process on chemical profiles and energy values of canola meal for dairy cattle;
- To determine the effects of temperature and time of conditioning during the pelleting process on nutrient availability of canola meal for dairy cattle;
- To determine pelleting-induced inherent molecular structural changes of protein and carbohydrate in canola meal using univariate and multivariate spectral techniques;
- To identify the differences in protein structure among raw (control) and pelleted canola meal;
- To quantify changes in molecular structure in relation to digestion characteristics and truly absorbed protein of canola meal and their pelleted products;
- To develop prediction equations of nutrient values based on spectral profiles of pelleted canola meal.

2.7.2. Hypotheses

The hypotheses for this study were: 1) sensitivity and responses of canola meal to different temperatures and time of conditioning are different; 2) the unprocessed canola meal differs from pelleted canola meal in nutrient profiles, digestion characteristics and predicted nutrient supply for dairy cattle; and, 3) processing-induced inherent structural changes affect digestion characteristics and nutrient supply of canola meal for dairy cattle.

3. Effects of Temperature and Time of Conditioning during the Pelleting Process on Chemical Profiles, Protein and Carbohydrate Subfractions, Rumen Degradation and Intestinal Digestion Characteristics, and Predicted Nutrient Supply of Canola Meal in Dairy Cattle

3.1. Introduction

Canola, which was bred from traditional rapeseed (*Brassica napus* and *Brassica campestris / rapa*) by Canadian plant breeders in the 1970s, is a high-oil-containing oilseed crop with low levels of erucic acid in the oil portion and low levels of glucosinolates in the meal portion (Bell, 1993; Heendeniya, 2008; Newkirk, 2009). The canola oil, which accounts for 42-43% of the seed, has served as a popular edible vegetable oil (Heendeniya, 2008; Newkirk, 2009). Canola meal, a co-product of oil extraction from canola seeds, has a high protein content and has been widely used as a protein source for animals (Yu, 2007; Newkirk, 2009). Although, canola meal is limited in lysine content (5.56% CP), its methionine (2.06% CP) and cysteine (2.39% CP) contents are relative high (Newkirk, 2009). Canola and rapeseed meals are considered as the second most traded protein source in the feed market (Heendeniya, 2008; Newkirk, 2009).

Pelleting, defined in 2.4.1, has been widely used in the animal feed industry and proved advantageous in improving feed quality (Skoch et al., 1981; Falk, 1985; Tamminga and Goelema, 1995; Thomas and van der Poel, 1996; Thomas et al., 1997; Abdollahi et al., 2013). Benefits and improvements provided by pelleting were outlined in 2.4.3.

Van der Poel et al. (1995) reported that degradation and passage rates of feeds through animals' digestive systems can be affected by feed processing methods. It was indicated that pelleting may improve starch availability for rumen degradation by 15% (Tamminga and Goelema, 1995; Thomas and van der Poel, 1996). However, the effects of pelleting process on feed materials should take into consideration the interrelations between conditioning, pelleting, and cooling (Thomas et al., 1997).

The production of Canadian canola was over 15 million tonnes in 2013 (Canola Council of Canada, 2013). In order to improve the competitiveness of canola meal in the feed market, it is necessary to develop suitable canola meal-based products with high feed values and transportation efficiency, as well as improved or undamaged nutrient quality. Although the nutritional values of canola meal have been studied for decades, information about the

effects of temperature and time of conditioning during the pelleting process in relation to nutritive quality and consistency between mash and pelleted canola meal is insufficient. This situation will be an obstacle to improving the quality of canola meal products, and will also affect the accuracy of diet formulation. Understanding how rumen degradation kinetics and intestinal digestion characteristics, and predicted nutrient supply, are affected by processing conditions is vital for feed evaluation. Hence, it is of interest to investigate the effects that pelleting under different conditioning temperatures and time has on nutrient profiles and digestion characteristics of canola meal, while trying to find the optimal processing conditions. The objectives of this study were: 1) to determine the effects of conditioning temperature and time on chemical and energy profiles of canola meal; 2) to investigate pelleting-induced changes in rumen degradation kinetics and intestinal digestion characteristics of canola meal in dairy cattle; 3) to detect pelleting-induced changes in predicted truly absorbed protein supply of canola meal for dairy cattle based on the NRC 2001 model.

3.2. Materials and Methods

3.2.1. Pelleting process and pellet durability test.

Canola meal samples from two different sources were collected in a commercial feed company (Federated Cooperatives Limited, Saskatoon, SK, Canada) with different manufacturing dates (January 9th and January 18th, 2013). Unprocessed canola meal was considered as control (raw). A California laboratory pellet mill (NH-397202, California Pellet Mill Co., Crawfordsville, IN, USA) with a capacity of 20 kg/h was used to produce canola meal pellets. The conditioning temperature (70, 80 and 90ºC) was set with heating pads wrapped around the conditioning chamber, and maintained with an Omega CN350 controller. The temperature of the conditioned mash was measured continuously at the outlet of the conditioner by an OMEGA HH-25TC thermometer (OMEGA Engineering Inc., Stamford, CT, USA). Conditioning time was determined by changing the flowrate at which the canola meal entered the conditioner. At a dial setting of “24” on the pelleting machine, the transition time of materials in the conditioner was 75 sec and 313 g of product were produced (250 g/min). At a dial setting of “30” on the pelleting machine, the conditioning time was changed to 50 sec and 665 g of product were produced during this transition time (814 g/min). Therefore, conditioning time is related to both flowrate and volume of feed exposed in the conditioner. The conditioning time was determined by placing colored materials into the

conditioner, then measuring time until the materials exited the conditioner. Conditioning temperature and time were combined in the experiment. Steam was injected with level of 1.0 kg/h at 36 psi during the conditioning stage. A pellet die with a hole diameter of 6.4 mm and a thickness of 44 mm was used. Pellets were cooled before the pellet durability test by exposing pellets in the air for 24 hours. The pellet durability was presented as a Pellet Durability Index (PDI), which was measured using the "Pfost" method and expressed on a percentage basis (Thomas and van der poel, 1996). Around 500 g of sieved pellets were put in a drum with specified dimensions of 30.5 cm × 30.5 cm × 12.7 cm, and then tumbled for 10 min at 50 rpm. After tumbling, the pellets were screened with a sieve in grid size to determine the pellets which could not pass through sieve. PDI was calculated as follows:

PDI = (weight of pellet before tumbling) / (weight of pellets after tumbling) × 100%

(adapted from Thomas and van der poel, 1996).

3.2.2. Animal and diets

Five lactating Holstein cows were used in the *in situ* animal trial for evaluating the degradation kinetics of canola meal pellets. Each cow was fitted with a rumen cannula with an internal diameter of 10 cm (Bar Diamond, Parma, ID, USA). The cows were cared for following the guidance of the Canadian Council on Animal Care (1993). The cows were fed a total mixed ration (TMR) twice daily at 0800 and 1600. Water was given *ad libitum*. The TMR feed was formulated with 56.1% barley silage, 14.3% chopped alfalfa and 29.6% concentrate to meet the NRC maintenance requirement (NRC, 2001).

3.2.3. Chemical analysis

The methods used in chemical analysis were reported in previous studies (Heendeniya et al., 2012; Yang et al., 2013a). For chemical analysis, all samples were ground through a 1 mm screen using a Retsch ZM 200 Rotor mill (Rose Scientific Ltd., Edmonton, AB, Canada). Dry matter (DM), ash, crude fat (CF), and crude protein (CP) were analyzed according to AOAC official methods 930.15, 942.05, 954.02, and 984.13, respectively. The methods described in Van Soest et al. (1991) combined with an ANKOM A200 filter bag technique (ANKOM Technology Corp., Fairport, NY, USA) were used to determine acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) in the samples. Adjusted NDF was determined based on Van Soest et al. (1991), combined with an ANKOM A200 filter bag technique, while sodium sulfite was added together with heat-stable amylase

before neutral detergent extraction. The methods in Licitra et al. (1996) were applied to analyze acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP). All samples were incubated with a bicarbonate-phosphate buffer, then filtered through the #54 Whatman filter papers to determine the total soluble crude protein (SCP) content (Roe et al., 1990). The method used to determine non-protein nitrogen (NPN) was in accordance with Licitra et al. (1996), in which tungstic acid is used to precipitate the true protein fraction and then the difference between total crude protein and precipitated crude protein is considered the NPN.

3.2.4. Estimation of energy values.

Summative approaches from the National Research Council (NRC, 2001) dairy were used to estimate values of the truly digestible non-fiber carbohydrates (tdNFC), the total digestible crude protein (tdCP), the total digestible neutral detergent fiber (tdNDF), the total digestible fatty acid (tdFA), the total digestible nutrients at 1x maintenance (TDN_{1x}), the total digestible nutrients at 3x maintenance (TDN_{3x}), the digestible energy (DE_{1x}), the digestible energy at the production level of 3x maintenance (DE_{p3x}), the metabolizable energy at the production level of 3x maintenance (ME_{p3x}), and the net energy at the production level of 3x maintenance (NE_{Lp3x}). The methods to determine the metabolizable energy (ME), the net energy for maintenance (NE_m) and the net energy for gain (NE_g) were in accordance with NRC (1996).

3.2.5. Profiling protein and carbohydrates fractions.

In this study, the Cornell Net Carbohydrate and Protein System (CNCPS) v.6.1 was applied to partition the CP and carbohydrate (CHO) fractions (Lanzas et al., 2007; Tylutki et al., 2008; Van Amburgh et al., 2010; Higgs et al., 2012). In the CNCPS v6.1, protein subfractions are profiled based on their ruminal degradation characteristics: ammonia (PA1), non-ammonia soluble true protein (PA2), moderately degradable true protein (PB1), slowly degradable true protein (PB2) and completely undegradable CP (PC) (Higgs et al., 2012; Van Amburgh et al., 2010, 2013). The CHO fraction scheme is expanded into eight subfractions: volatile fatty acids (VFA) (CA1), lactate (CA2), organic acids (CA3), sugars (CA4), starch (CB1), soluble fibers (CB2), slowly degradable and available NDF (CB3) and lignin (CC) (Tylutki et al., 2008; Van Amburgh et al., 2010; Higgs et al., 2012; Van Amburgh et al., 2013). Fractional degradation and passage rates were obtained from the CNCPS feed library

and literature (Lanzas et al., 2007; Tylutki et al. 2008; Van Amburgh et al., 2010; Higgs et al., 2012). In canola meal, CA1, CA2, and CA3 are not presented, and only a small amount of CB1 (5%) was found (Newkirk, 2009). Hence, CHO subfractions focused on in this study were CA4, CB2, CB3, and CC.

3.2.6. Rumen incubation and residual chemical analysis

The *in situ* method described in Yu et al. (2000) was used to conduct the rumen incubation. A roller mill (Apollo Machine & Products Ltd., Saskatoon, SK, Canada) with a roller gap of 0.203 mm was used to coarsely grind samples. Around 7 g of ground samples were weighed and put in numbered nylon bags with 40 μm pores (Nitex 03-41/31 monofilament open mash fabric, Screentec Corp., Mississauga, ON). The bags were then tied and randomly assigned to five polyester mesh bags and suspended in the rumen The nylon bags in each cow did not exceed 30. All samples were randomly carried out in five rumen-cannulated cows in two runs for each incubation period. For a long incubation period, the number of bags was increased to ensure enough residues for chemical analysis after incubation. Ruminal incubation processes were carried out for 0, 2, 4, 8, 12, 24, and 48 h, according to the "gradual addition/all out" schedule (Yu et al., 2000). At the end of incubation, bags were removed from the rumen and then hand washed along with 0 h bags in detergent-free cold water to rinse off ruminal contents. Washed bags were then dried in a forced air oven at 55ºC for 48 h. The dry residual samples in bags were pooled according to the sequence of treatments, incubation time, and run. Then pooled samples were ground through a 1 mm screen using a Retsch ZM 200 rotor mill and then prepared for residual chemical analysis. DM and ash contents were analyzed using the AOAC official procedures. The CP content was detected using Leco Protein/N Analyzer FP-528 (Leco Corp., St Joseph, MI, USA). The NDF content was determined with an ANKOM A200 filter bag technique based on Van Soest et al. (1991). All the samples were analyzed in duplicate, and reprocessed when the error was larger than 5%.

3.2.7. Rumen degradation kinetics

The first order kinetics degradation model (Ørskov and McDonald, 1979), and modified by Robinson et al. (1986) and Dhanoa (1988), with lag time included was used to determine the degradation kinetics of DM, CP, NDF, and CHO, as follows:

$$R(t) = U + D \times e^{-Kd \times (t-T0)},$$

where, R(t) is residuals of samples after t h incubation; U means rumen undegradable fraction of samples; D is rumen degradable fraction of samples, respectively (%); Kd is the degradation rate (%/h); t means incubation time; and T_0 is lag time (h).

The data in the model was processed using the NLIN (nonlinear) procedure of SAS 9.3, with iterative least squares regression (Gauss Newton method). Based on the parameters calculated above, the effectively degraded (ED) and rumen undegraded (RU) fractions of DM, CP, NDF, and CHO was calculated using equations in the NRC 2001:

$$ED = S + D \times [Kd / Kp + Kd)];$$

$$RU = U + D \times [Kp / (Kp + Kd)].$$

Where, S means soluble fraction (%) and Kp represents the passage rate of digesta from the rumen (6%/h for concentrates, 4.5%/h for roughages, and 4%/h for forages) (Tamminga et al., 1994).

3.2.8. Hourly effective degradation ratios and the potential N-to-energy synchronization

The amount of nutrients degraded each hour can be ascertained by calculating the difference in cumulative hourly ED amount between successive hours. Equations in Sinclair et al. (1993) were derived to calculate hourly effectively degraded nitrogen (N) and OM:

$$\text{Hourly ED (g/kg DM)} = S + [(D \times K_d) / (K_p + K_d)] \times [1 - e^{-t \times (Kd + Kp)}].$$

Hourly effective degradation ratio of N to OM and hourly effective degradation ratio of N to CHO were calculated using the equations from Nuez-Ortín and Yu (2010) and Yang et al. (2013a), as follows:

$$\text{Hourly ED ratio } N/OM_t = (HEDN_t - HEDN_{t-1}) / (HEDOM_t - HEDOM_{t-1});$$

$$\text{Hourly ED ratio } N/CHO_t = 1000 \times (HEDN_t - HEDN_{t-1}) / [(HEDNDF_t - HEDNDF_{t-1}) + (HEDNFC_t - HEDNFC_{t-1})].$$

Where, N/OM_t is the ratio of N to OM at time t (g N/kg OM); HEDNt means hourly effective degradability of N at time t (g/kg DM); $HEDN_{t-1}$ represents hourly effective degradability of N at 1 h before t (g/kg DM); $HEDOM_t$ means hourly effective degradability of OM at time t (g/kg DM); $HEDOM_{t-1}$ is hourly effective degradability of OM at 1 h before t (g/kg DM); N/CHO_t means the ratio of N to CHO at time t (g N/kg CHO); $HEDCHO_t$ is hourly effective degradability of CHO at time t (g/kg DM); $HEDNDF_t$ is hourly effective degradability of NDF at time t (g/kg DM); $HEDNDF_{t-1}$ represents hourly effective degradability of NDF at 1 h before t (g/kg DM); $HEDNFC_t$ is hourly effective degradability

of non-fiber carbohydrate (NFC) at time t (g/kg DM); $HEDNFC_{t-1}$ equals hourly effective degradability of NFC at 1 h before t (g/kg DM).

The optimal ratios of N/OM and N/CHO are 25 g N/kg OM and 32 g N/kg CHO truly digested in the rumen, respectively (Czerkawski, 1986; Tamminga et al., 1990; Sinclair et al., 1991; Sinclair et al., 1993).

3.2.9. Intestinal digestion of nutrients

In the *in vitro* study, a three-step *in vitro* procedure reported in Calsamiglia and Stern (1995) was employed to detect intestinal digestion of rumen undegraded protein (RUP). Around 15 g of ground dry samples were derived from rumen residues incubated for 12 h. Then, samples were mixed with 10 mL 0.1 mol/L HCl containing 1 g/L pepsin, and incubated for 1 h at 38ºC. After incubation, the solution was neutralized with 0.5 mL of 1 mol/L NaOH and 13.5 mL phosphate buffer containing 37.5 mg pancreatin (pH = 7.8), then incubated for 24 h at 38 ºC. During 24 h of incubation, tubes were taken out and vortexed every 8 h. After incubation, 3 mL of 100 % Trichloroacetic acid (TCA) solution was added into the mixture to stop enzymatic activity and precipitate the undigested protein. The supernatant after centrifuging was taken for N analysis using the Kjeldahl method (AOAC, 984.13). The ratio of TCA-soluble N to N in the sample expressed on a percentage basis is considered the digestibility of protein in the small intestine (Calsamiglia and Stern, 1995; NRC, 2001; Gargallo et al., 2006; Nuez-Ortín and Yu, 2010). In addition, the intestinal digestibility of CHO, NDF, and DM were also predicted (Calsamiglia and Stern, 1995; Nuez-Ortín and Yu, 2010; Yang et al., 2013a).

3.2.10. Prediction of nutrient supply and availability to dairy cattle based on the NRC 2001 model

Estimations in the NRC 2001 model are based on the TDN. In addition, endogenous protein is considered a gain in the small intestine. The NRC 2001 model calculates metabolizable protein (MP) as the sum of truly absorbable rumen degradable protein in the small intestine ($ARUP^{NRC}$), truly absorbable rumen synthesized microbial protein in the small intestine ($AMCP^{NRC}$), and absorbable endogenous protein passed from rumen to the small intestine (AECP) (NRC, 2001; Yu, 2005a). All the variables required to obtain the MP value can be calculated based on summative formulas in NRC (2001). Degraded protein balance (OEB^{NRC}) was then calculated based on data from the NRC 2001 model, as follows:

$$OEB^{NRC}\ (g/kg\ DM) = RDP^{NRC} - 1.18 \times MCP_{TDN},$$

Where, RDP^{NRC} is rumen degradable protein; 1.18 means 1/0.85, used to convert MCP_{TDN} to RDP; MCP_{TDN} is microbial protein synthesized in the rumen based on TDN_{3x}.

3.2.11. Feed milk value (FMV)

With regards to the nutrient values of protein, the rumen synthesized microbial protein and the intestinal digested protein were used as a dietary N source for milk production. The feed milk value (FMV) was used to determine response and efficiency of dietary N for milk production (Theodoridou and Yu, 2013b). The FMV was calculated according to NRC (2001), as follows:

$$FMV^{NRC}\ (kg/kg\ DM) = MP^{NRC}\ (g/kg\ DM) \times 0.67 / 33,$$

where, 0.67 means efficiency of MP for lactation and 33 means 1000 g milk is assumed to contain 33 g protein (NRC, 2001; Theodoridou and Yu, 2013b).

3.2.12. Statistical analysis

This study was designed using the randomized complete block design (RCBD) with a 3 × 2 factorial arrangement. Statistical analyses were performed using the MIXED procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). For chemical analysis, nutrient fractions, and energy estimation, the following model was used:

$$Y_{ijr} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijr},$$

in which Y_{ijr} was an observation of the dependent variable *ijr*, μ was the population mean for the variable, α_i was the effect of conditioning temperature, β_j was the effect of conditioning time, γ_k was the interaction of conditioning time and temperature, and e_{ijr} was the random error associated with observation *ijr*.

For the analyses of the *in situ* degradation kinetics and *in vitro* digestion characteristics of nutrients as well as predicted nutrient supply, the following formula was used:

$$Y_{ijlr} = \mu + \alpha_i + \beta_j + \gamma_k + \eta_l + e_{ijlr},$$

where Y_{ijlr} was an observation of the dependent variable *ijlr*, μ was the population mean for the variable, α_i was the effect of conditioning temperature, β_j was the effect of conditioning time, γ_k was the interaction of conditioning time and temperature, η_l was the effect of experiment run as a random effect, and e_{ijlr} was random errors associated with observation *ijlr*.

A contrast statement was performed to detect the difference between the unprocessed mash (raw) and pellets. Also, polynomial contrasts (linear and quadratic) of conditioning temperature (70, 80 and 90ºC) were determined if the linear effect of conditioning temperature was significant. In the current study, the significance level for all statistical analyses was declared at $P<0.05$ and trends at $P<0.10$. Treatment means were compared using the Tukey-Kramer method.

3.3. Results and Discussion

3.3.1. Effect of temperature and time of conditioning during the pelleting process on canola meals, in terms of the pellet durability index (PDI)

Shearing and abrasion happen during pellet manufacturing. This can damage pellets, potentially turning them into mash. Hence, pellets must have a sound resistance against stress during transportation and distribution (Thomas and van de Poel, 1996). The results of pellet durability tests are shown in Table 3.1. The interaction of conditioning temperature and time on PDI of canola meal pellets was not significant ($P>0.05$). The PDI of canola meal pellets was not affected by conditioning temperature ($P>0.05$), but was positively affected by conditioning time ($P<0.05$). Samples conditioned for 75 sec had a higher PDI than those conditioned for 50 sec (91.3 vs. 73.4%). Previous studies indicated that increasing conditioning time would improve PDI (Thomas and van der Poel, 1996; Thomas et al., 1997; Abdollahi et al., 2013). Abdollahi et al. (2013) indicated the pellet quality would be improved if diets had a high protein content, and/or included fiber. Increasing the retention time of mash feed in the conditioner, along with injection of steam, contributed to the forming of hydrogen bonds between hydrophilic portions of protein and water molecules (Abdollahi et al., 2013). In addition, longer conditioning time could take advantage of the increased thermo-mechanical nutrient interaction and enhanced feed adhesion to improve pellet durability (Fairchild and Greer, 1999; Moritz and Lilly, 2010; Abdollahi et al., 2013).

Table 3.1. Effects of conditioning temperature and time during pelleting process on pellet durability index of canola meal pellets

Item	Conditioning Temperature (Temp)			SEM	Conditioning Time (time)		SEM	P value			Polynomial contrast for Temp (P value)	
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)		50 sec (n=6)	75 sec (n=6)		Temp	Time	Temp×Time	L	Q
PDI (%)	85.3	81.2	80.3	5.50	73.4	91.3	4.49	0.80	0.03	0.83	0.54	0.85

Notes: PDI: pellet durability index; SEM: standard error of means; L: Linear; Q: Quadratic; Means with different letters in the same row are significantly different.

3.3.2. Effects of temperature and time of conditioning during the pelleting process on chemical profiles, estimated energy values, and protein and carbohydrate subfractions of canola meal

3.3.2.1. Effects of temperature and time of conditioning during the pelleting process on chemical profiles of canola meal

Table 3.2 shows the effects of pelleting under different conditions on the chemical profiles of canola meal. The interaction of conditioning temperature and time was not significant on the basic chemical profiles ($P>0.05$). The conditioning temperature and time did not exhibit a significant effect on basic chemical profiles (ash, OM, and EE) ($P>0.05$). For carbohydrate profiles, NDF (30.5% DM), ADF (19.4% DM), and ADL (8.1% DM) were not affected by different conditioning temperatures and time ($P>0.05$). Total CP content of canola meal pellets with an average 39.9% DM was not affected by conditioning temperature and time ($P>0.05$). Samadi and Yu (2011) reported that heating methods would not influence total CP content. McKinnon et al. (1995) indicated that the total CP content did not change when processed under different heating temperatures and time. However, significant effects of conditioning time on SCP ($P<0.05$) and NDICP ($P<0.01$) were detected in our study. Samples conditioned for 75 sec had a lower SCP content and a higher NDCIP content than those conditioned for 50 sec. However, the unprocessed mash was different from the pelleted samples ($P<0.05$) with higher NDICP. Goelema (1999) summarized that heating can stabilize protein structure and create crosslinks between proteins and carbohydrates. In addition, protein is very sensitive to protein environmental temperature. Any change in temperature, which is able to affect the non-covalent interaction in protein structure, may induce changes in the quaternary, tertiary and/or secondary structures of the protein (Goelema, 1999). It was reported that not only processing temperature but also processing time during heat treatment play important roles in determining the level of denaturation and degradation of protein (Goelema, 1999). During the conditioning stage, increasing conditioning time not only hydrates materials but also reduces friction when passed through the pellet die (Thomas et al., 1997; Abdollahi et al., 2013). The ideal heating treatment was able to increase NDICP, reduce SCP of feed, while the increase in ADICP was minimized at the same time (Van Soest, 1989).

Table 3.2 Effects of temperature and time of conditioning during the pelleting process on chemical profiles of canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)		Control	Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	(Raw) (n=2)	Control vs. processed
Basic chemical profiles														
Ash (%DM)	7.2	7.2	7.2	0.07	7.2	7.2	0.07	0.70	0.82	0.73	0.50	0.64	7.3	0.18
DM (%)	87.0	89.2	87.3	1.46	87.6	88.1	1.19	0.55	0.78	0.25	0.89	0.30	91.0	0.20
OM (%DM)	92.8	92.8	92.8	0.07	92.8	92.8	0.07	0.70	0.82	0.73	0.50	0.64	92.8	0.18
EE (%DM)	1.7	1.8	1.7	0.20	1.7	1.8	0.19	0.85	0.35	0.44	0.75	0.66	1.5	0.30
Carbohydrate profiles														
NDF (%DM)	30.5	30.5	30.5	0.89	30.5	30.8	0.88	1.00	0.77	0.93	0.95	0.99	30.2	0.48
ADF (%DM)	19.7	19.4	19.4	1.12	19.4	19.6	1.11	0.43	0.28	0.73	0.25	0.59	19.1	0.14
ADL (%DM)	8.2	8.4	8.3	0.36	8.2	8.4	0.35	0.37	0.10	0.18	0.68	0.19	8.1	0.38
ADL (%NDF)	26.8	27.5	27.0	0.49	26.8	27.5	0.44	0.50	0.20	0.32	0.72	0.28	27.0	0.82
CHO(%DM)	51.1	51.2	51.2	1.08	51.3	51.0	1.07	0.94	0.33	0.81	0.75	0.92	51.9	0.15
Crude protein profiles														
CP (%DM)	40.1	39.9	39.9	1.19	39.8	40.1	1.17	0.86	0.56	0.98	0.64	0.82	39.4	0.24
SCP (%CP)	20.7	21.1	21.0	0.57	21.7	20.1	0.51	0.79	0.03	0.40	0.62	0.67	21.0	0.89
NPN (%SCP)	81.9	78.8	81.3	3.63	79.0	82.3	3.46	0.52	0.19	0.35	0.83	0.28	80.1	0.85
NDICP (%CP)	16.2	16.2	16.4	0.46	16.0	16.5	0.46	0.63	0.03	0.38	0.48	0.55	17.2	0.02
NDICP (%DM)	6.5	6.5	6.5	0.03	6.4	6.6	0.03	0.42	<0.01	0.14	0.68	0.22	6.7	0.03
ADICP (%DM)	1.8	1.7	1.8	0.64	1.7	1.8	0.06	0.58	0.20	0.09	0.71	0.35	1.8	0.77
ADICP (% CP)	4.4	4.3	4.5	0.28	4.3	4.5	0.27	0.70	0.34	0.19	0.70	0.48	4.6	0.59

Notes: SEM: Standard error of means. L: Linear. Q: Quadratic. DM: dry matter; CP: crude protein; NDF: neutral detergent fiber, with ash; ADF: acid detergent fiber, with ash; ADL: acid detergent lignin; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; NPN: non-protein nitrogen; SCP: soluble crude protein; EE: ether extracts (crude fat); NPN: non-protein nitrogen; OM: organic matter. Means with different letters in the same row are significantly different (P<0.05).

McKinnon et al. (1995) observed that reduced SCP and increased NDICP contents were associated with increased NDF content when processing time was increased during dry heating of canola meal at 125 and 145ºC. In the current study, samples conditioned for 50 sec tended to have a lower NDF content than those conditioned for 75 sec ($P<0.10$). Reduced soluble protein and increased NDICP contents were likely due to the formation of crosslinks between protein and fiber during the pelleting process (Goelema, 1999)

The ADICP content of samples with an average of 4.5 in %CP was not affected in the current study. McKinnon et al. (1995) observed increased ADICP and NDICP when canola meal was heated from 125 to 145ºC. They assumed that increased processing temperature was responsible for this increase. Nia and Ingalls (1992) observed ADICP and NDICP were increased by increasing heating time during autoclaving at 127ºC. Mustafa et al. (2000) indicated that NDICP and ADICP were increased and SCP was decreased after desolventizing-toasting (103 to 107ºC). In addition, ADICP content can reflect heat-damaged indigestible protein in feed materials (McKinnon et al., 1995). McKinnon et al. (1995) also observed decreased intestinal CP digestibility because of increased ADICP in canola meal. During canola processing, canola meal has been heated up to 120ºC, which results in protein denaturation and the Maillard reactions (Newkirk, 2009). Canola protein was not damaged further in our study.

3.3.2.2. Effects of temperature and time of conditioning during the pelleting process on energy values of canola meal

Effects of temperature and time of conditioning during the pelleting process on energy values of canola meal are presented in Table 3.3. To summarize, energy values were not affected by conditioning temperature and time. No significant effect of conditioning time, temperature, or their interaction on truly digestible nutrients (tdNFC, tdCP, tdNDF, and tdFA) was found, but tdNFC of the unprocessed mash tended to be higher than that of pellets (27.8 vs. 26.6% DM; $P<0.10$). Total digestible nutrients (TDN_{1x}) and predicted energy values (DE_{1x}, DE_{p3x}, ME_{p3x}, NE_{Lp3x}, ME, NE_m, and NE_g) were not affected by the pelleting process in the current study ($P>0.05$).

Table 3.3 Effects of temperature and time of conditioning during the pelleting process on energy values of canola meal for dairy and beef cattle

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
Truly digestible nutrients														
tdNFC, %DM	26.5	26.6	26.6	0.43	26.9	26.4	0.35	0.96	0.63	0.91	0.78	0.99	27.8	0.08
tdCP, %DM	39.4	39.2	39.2	1.21	39.1	39.3	1.19	0.88	0.63	1.00	0.64	0.87	38.6	0.26
tdNDF, %DM	6.1	6.0	6.0	0.22	6.2	5.9	0.20	0.80	0.21	0.67	0.77	0.57	5.9	0.51
tdFA, %DM	0.7	0.8	0.7	0.20	0.7	0.8	0.19	0.85	0.35	0.44	0.75	0.66	0.5	0.30
Total digestible nutrients														
TDN_{1x}, %DM	66.4	66.4	66.4	0.47	66.4	66.5	0.45	0.99	0.91	0.51	1.00	0.89	66.5	0.92
Predicted energy values														
DE_{1x}, Mcal/kg, dairy	3.33	3.33	3.33	0.036	3.33	3.34	0.037	1.00	0.51	0.23	1.00	1.00	3.33	0.45
DE_{p3x}, Mcal/kg, dairy	3.17	3.17	3.16	0.027	3.16	3.17	0.027	0.78	0.58	0.39	0.50	1.00	3.16	0.49
ME_{p3x}, Mcal/kg, dairy	2.75	2.75	2.75	0.026	2.75	2.75	0.026	0.71	0.34	0.40	0.69	0.49	2.74	0.26
NE_{Lp3x}, Mcal/kg, dairy	1.74	1.74	1.74	0.019	1.74	1.75	0.018	0.86	0.17	0.40	1.00	0.60	1.74	0.29
ME, Mcal/kg, beef	2.74	2.73	2.73	0.029	2.73	2.74	0.029	0.77	0.71	0.44	0.50	0.89	2.73	0.46
NE_m, Mcal/kg, beef	1.81	1.80	1.81	0.025	1.81	1.81	0.025	0.59	1.00	0.27	0.74	0.35	1.80	0.39
NE_g, Mcal/kg, beef	1.18	1.18	1.18	0.021	1.18	1.18	0.021	0.93	0.79	0.61	1.00	0.71	1.18	0.60

Notes: SEM: Standard error of means; tdNFC: truly digestible non-fiber carbohydrates; tdCP: total digestible crude protein; tdNDF: total digestible neutral detergent fiber; tdFA: total digestible fatty acid; TDN_{1x}: total digestible nutrients; DE_{1x}: digestible energy; DE_{p3x}: digestible energy at a production level (3x maintenance); ME_{p3x}: metabolizable energy at a production level (3x maintenance); NE_{Lp3x}: net energy at a production level (3x maintenance); ME: metabolizable energy; NE_m: net energy for maintenance; NE_g: net energy for gain; L: Linear; Q: Quadratic; Means with different letters in the same row are significantly different.

3.3.2.3. Effects of temperature and time of conditioning during the pelleting process on protein and carbohydrate profiles of canola meal

Models in CNCPS v6.1 are more sensitive to the feeds with high levels of protein and carbohydrates (Higgs et al., 2012; Van Amburgh et al., 2010, 2013). For example, in order to get a more appropriate reflection of cattle biology in soluble pools, re-assignation of the liquid passage rate equations to CHO A, PA, and PB has been made. The resultant higher predicted outflow of soluble components would reduce estimated microbial yield and ammonia production (Van Amburgh et al., 2010).

Table 3.4 shows the CHO and protein subfractions of all samples. Conditioning time had a negative effect on PA2 fraction ($P<0.05$). Samples conditioned for 50 sec exhibited a higher PA2 content than those conditioned for 75 sec (21.7 vs. 20.1% CP). Samples conditioned for 75 sec tended toward a higher PB1 content than those conditioned for 50 sec (63.4 vs. 62.3% CP, $P<0.10$). The PB2 was significantly affected by the interaction of conditioning time and temperature ($P<0.05$). At conditioning time of 50 sec, samples conditioned at 70ºC were not different from those conditioned at 80ºC in PB2, but samples conditioned 90ºC had higher PB2 than those conditioned at 80ºC. At conditioning time of 75 sec, samples conditioned at 70ºC were not different from those conditioned at 80 or 90ºC in PB2. However, samples conditioned at 80ºC were higher in PB2 than those conditioned at 90ºC (Figure 3.1). The average PB2 of pellets were lower than that of unprocessed mash ($P<0.05$), demonstrating that the pelleting with relative low temperatures negatively affected PB2. There was no difference in PC content among all samples ($P>0.05$).

The total CHO content of canola meal was not affected when pelleting under different conditioning temperatures and time. No significant effect of conditioning temperature, time, or their interaction on carbohydrate subfractions, in terms of CA4, CB2, CB3, and CC, was found ($P>0.05$).

Table 3.4 Effects of temperature and time of conditioning during pelleting process on protein and carbohydrate subfractions in canola meal using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.1

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)		Control	Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	(Raw) (n=2)	Control vs. processed
Protein subfractions														
PA2 (%CP)	20.7	21.1	21.1	0.57	21.7	21.1	0.51	0.79	0.03	0.39	0.62	0.67	21.0	0.89
PB1 (%CP)	63.2	62.8	62.9	0.46	62.3	63.4	0.37	0.76	0.09	0.53	0.50	0.81	61.9	0.22
PB2(%CP)	11.8	11.9	11.9	0.22	11.7	12.0	0.21	0.87	0.08	0.03	0.72	0.72	12.6	<0.01
PC (%CP)	4.4	4.3	4.5	0.28	4.3	4.5	0.27	0.70	0.34	0.19	0.70	0.48	4.6	0.59
Carbohydrate subfractions														
CHO(%DM)	51.1	51.2	51.2	1.08	51.3	51.0	1.07	0.94	0.33	0.81	0.75	0.92	51.9	0.15
CA4 (%CHO)	23.1	21.4	21.2	0.65	2.1	21.7	0.53	0.16	0.56	0.30	0.09	0.38	22.1	0.84
CB2 (%CHO)	17.4	17.3	20.3	1.73	20.0	16.6	1.41	0.45	0.15	0.22	0.30	0.50	22.1	0.17
CB3 (%CHO)	21.0	21.9	19.9	1.11	19.7	22.2	0.91	0.49	0.11	0.47	0.52	0.33	18.2	0.13
CC (%CHO)	38.5	39.4	38.7	0.97	38.2	39.5	0.90	0.58	0.12	0.37	0.85	0.33	37.7	0.25

Notes: SEM: Standard error of means. PA2: rapidly degradable true protein; PB1: moderately degradable true protein; PB2: slowly degradable true protein; PC: undegradable protein; CHO: carbohydrates; CA4: sugars; CB2: soluble fibers; CB3: available nNDF; CC: unavailable cell walls; L: Linear; Q: Quadratic. Means with different letters in the same row are significantly different (P<0.05).

Pelleted samples were not different from the unprocessed mash in regards to carbohydrate, either (P>0.05). Theodoridou and Yu (2013c) observed that CA and CB were decreased but CC was increased when canola presscake was desolventized-toasted. However, the pelleting under different conditioning temperatures and time had no effect on carbohydrate subfractions of canola meal in our study.

Effects of temperature and time of conditioning during the pelleting on rumen degradable and undegradable subfractions of protein and carbohydrate are shown in Table 3.5. The samples conditioned for 50 sec differed from those conditioned for 75 sec (P<0.05): higher rumen degradable PA2 (RDPA2) and rumen undegradable PA2 (RUPA2) (6.6 vs. 6.2% DM, 2.0 vs. 1.9% DM, respectively). No significant effect of conditioning temperature, time, or their interaction on rumen degraded and escaped subfractions of carbohydrates was observed (P>0.05). There was no significant difference among all samples in terms of subfractions of rumen degraded and escaped CHO (RDCHO and RUCHO), as well as total rumen degraded and escaped CHO (TRDC and TRUCC).

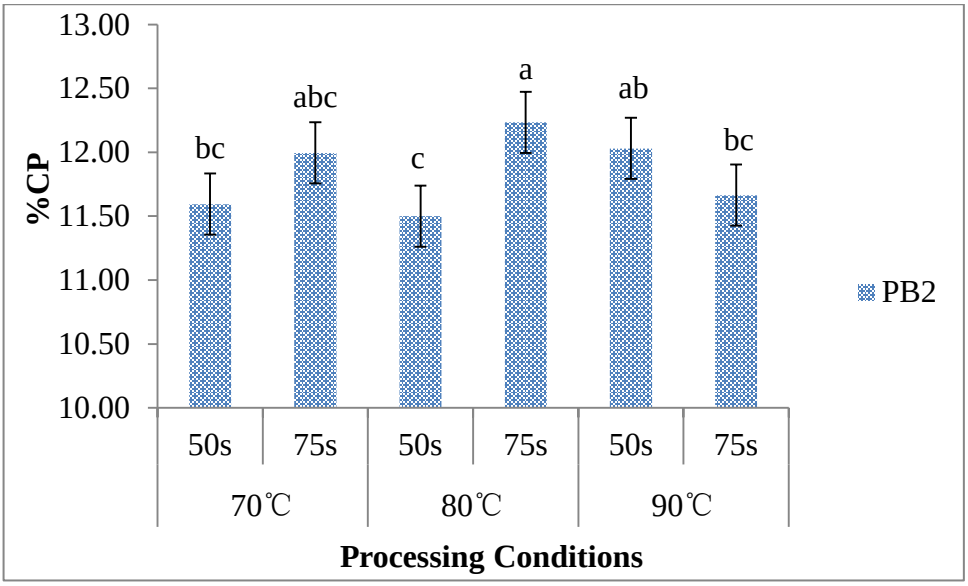


Figure 3.1 Effect of interaction of conditioning temperature and time on PB2 content of canola meal pellets. Bars with different letters are significantly different (n=12; P<0.05)

Table 3.5 Effects of temperature and time of conditioning during the pelleting process on rumen degradable and undegradable subfractions of protein and carbohydrates in canola meal using Cornell Net Carbohydrate and Protein System (CNCPS) v.6.1

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)		Control	Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	(Raw) (n=2)	Control vs. processed
Rumen degradable protein fractions														
RDPA2(%DM)	6.4	6.5	6.4	0.34	6.6	6.2	0.33	0.91	0.04	0.38	0.77	0.77	6.4	0.73
RDPB1(%DM)	17.7	17.5	17.5	0.55	17.4	17.8	0.53	0.75	0.17	0.85	0.50	0.79	17.1	0.15
RDPB2(%DM)	1.9	1.9	1.9	0.03	1.9	1.9	0.03	0.99	0.16	0.17	0.90	0.94	2.0	0.06
TRDP(%DM)	26.0	25.9	25.8	0.88	25.9	25.9	0.87	0.89	0.92	0.99	0.65	0.93	25.4	0.19
Rumen undegradable protein fractions														
RUPA2(%DM)	1.9	1.9	1.9	0.10	2.0	1.9	0.10	0.90	0.04	0.39	0.75	0.75	1.9	0.74
RUPB1(%DM)	7.6	7.5	7.5	0.23	7.4	7.6	0.23	0.75	0.17	0.85	0.49	0.79	7.3	0.15
RUPB2(%DM)	2.8	2.8	2.8	0.05	2.8	2.9	0.04	0.99	0.15	0.17	0.93	0.96	3.0	0.06
TRUP(%DM)	14.1	14.1	14.0	0.31	14.0	14.2	0.31	0.70	0.09	0.73	0.64	0.50	14.0	0.54
Rumen degradable carbohydrate fractions														
RDCA4(%DM)	9.1	8.4	8.3	0.23	8.7	8.5	0.19	0.13	0.37	0.26	0.07	0.36	8.8	0.56
RDCB2(%DM)	7.4	7.4	8. 7	0.75	8.6	7.1	0.61	0.45	0.15	0.24	0.28	0.52	9.5	0.15
RDCB3(%DM)	4.3	4.5	4.1	0.22	4.0	4.5	0.18	0.46	0.11	0.46	0.50	0.30	3.8	0.14
TRDC(%DM)	20.7	20.3	21.1	0.51	21.3	20.1	0.42	0.59	0.08	0.40	0.69	0.37	22.1	0.10
Rumen undegradable carbohydrate fractions														
RUCA4(%DM)	2.7	2.5	2.5	0.07	2.6	2.6	0.06	0.13	0.38	0.27	0.07	0.36	2.7	0.57
RUCB2(%DM)	1.5	1.5	1.7	0.15	1.7	1.4	0.12	0.45	0.15	0.24	0.29	0.51	1.5	0.16
RUCB3(%DM)	6.4	6.7	6.1	0.32	6.1	6.8	0.26	0.46	0.11	0.46	0.49	0.31	5.7	0.15
TRUCC(%DM)	30.3	30.9	30.1	0.99	30.0	30.9	0.97	0.31	0.06	0.17	0.74	0.15	29.8	0.20

Notes: SEM: Standard error of means. RDPA2: ruminally degraded PA2; RDPB1: ruminally degraded PB1; RDPB2: ruminally degraded PB2; TRDP: total ruminally degraded CP; RUPA2: ruminally escaped PA2; RUPB1: ruminally escaped PB1; RUPB2: ruminally escaped PB2; RUPC: ruminally escaped PC; TRUP: total ruminally escaped CP; RDCA4: ruminally degraded CA4; RDCB2: ruminally degraded CB2; RDCB3: ruminally degraded CB3; TRDC: total ruminally degraded CHO; RUCA4: ruminally escaped CA4; RUCB2: ruminally escaped CB2;

Table 3.5 Cont’d.

RUCB3: ruminally escaped CB3; RUCC: ruminally escaped CC; TRUCC: total ruminally escaped CHO; L: Linear; Q: Quadratic. Means with different letters in the same row are significantly different.

3.3.3. Effects of temperature and time of conditioning during the pelleting process on rumen degradation kinetics of nutrients of canola meal

3.3.3.1. *In situ* rumen degradation kinetics of DM

Table 3.6 shows the effect of conditioning temperature and time on rumen degradation kinetics of DM. Our study determined the interaction of conditioning time and temperature on degradation kinetics of DM was not significant (P>0.05). No significant effect of conditioning time on Kd for DM was observed (P>0.05). The samples conditioned at 80ºC tended to have the highest value of Kd (11.2%/h; P<0.10). McKinnon et al. (1995) found decreased DM degradation when canola meal was dry heated at 125 and 145ºC for 10, 20, and 30 min. In our study, The T0 of DM were not affected (P>0.05).

A positive impact of conditioning time on D of DM in pellets was detected (P<0.05). The samples conditioned for 75 sec had a higher D of DM than those conditioned for 50 sec (69.1 vs. 67.3% DM). The difference between the pelleted samples and the unprocessed mash in D of DM was not significant (P>0.05). There was no difference observed in U of DM among all samples. The samples conditioned at 80ºC tended to have the lowest rumen bypass DM (BDM) content (418 g/kg DM) but the highest rumen effectively degraded DM (EDDM) content (582 g/kg DM) compared with those conditioned at 70ºC and 90ºC (P<0.10). Nia and Ingalls (1992) observed reduced DM disappearance of canola meal in the rumen with increasing autoclaving time at 127ºC. Sadeghi and Shawrang (2006) observed S and D of DM in canola meal were decreased with increasing processing time during microwave radiation. In our study, rumen effective degradation of DM tended to be lowest at conditioning temperature of 90ºC (P<0.10).

3.3.3.2. *In situ* rumen degradation kinetics of CHO

Effects of temperature and time of conditioning on the *in situ* rumen degradation kinetics of CHO are presented in Table 3.7. It was noted that Kd, T0, and U of CHO was not affected by pelleting under different conditioning temperatures and time.

Table 3.6 Effects of temperature and time of conditioning during the pelleting process on *in situ* rumen degradation characteristics of dry matter in canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)		Control (Raw) (n=2)	Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q		Control vs. processed
In situ rumen degradation														
K_d (%/h)	9.5	11.2	9.2	0.59	10.0	9.9	0.48	0.06	0.80	0.77	0.73	0.02	10.4	0.67
T_0 (h)	0.1	0.3	0.1	0.16	0.2	0.1	0.14	0.31	0.89	0.71	0.94	0.13	0.0	0.43
S (%DM)	14.1	14.7	15.1	0.61	15.1	14.2	0.54	0.40	0.13	0.59	0.19	0.87	14.4	0.71
D (%DM)	68.7	67.5	68.4	1.85	67.3	69.1	1.81	0.44	0.04	0.89	0.81	0.22	67.9	0.86
U (%DM)	17.2	17.8	16.5	1.38	17.6	16.8	1.35	0.16	0.16	0.88	0.26	0.12	17.8	0.65
BDM (g/kg, DM)	440	418	436	10.3	432	430	9.65	0.07	0.77	0.99	0.65	0.02	444	0.23
EDDM (g/kg, DM)	560	582	564	10.3	568	570	9.7	0.06	0.77	0.99	0.65	0.02	556	0.23

Notes: SEM: Standard error of means. Kd: degradation rate of D fraction; T0: lag time in the rumen; S: soluble fraction in the in situ rumen incubation; D: potentially degradable fraction; U: rumen undegradable fraction; BDM: rumen bypass dry matter; EDDM: rumen effectively degraded dry matter; L: Linear; Q: quadratic. Means with different letters in the same row are significantly different.

Table 3.7 Effects of temperature and time of conditioning during the pelleting process on *in situ* rumen degradation characteristics of carbohydrates in canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)		Control (Raw) (n=2)	Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q		Control vs. processed
In situ rumen degradation														
K_d (%/h)	9.7	11.7	9.6	0.87	10.5	10.2	0.71	0.17	0.77	0.89	0.92	0.07	8.5	0.16
T_0 (h)	0.3	0.5	0.3	0.27	0.5	0.3	0.25	0.71	0.49	0.38	0.97	0.42	0.3	0.75
S (%CHO)	16.8	18.4	20.3	1.35	19.7	17.3	1.22	0.07	0.06	0.61	0.03	0.87	19.4	0.56
D (%CHO)	56.2	53.7	53.1	1.11	53.1	55.5	0.90	0.14	0.08	0.72	0.47	0.07	54.8	0.79
U (%CHO)	27.0	28.0	26.6	1.59	27.3	27.2	1.54	0.32	0.92	0.71	0.70	0.15	25.7	0.12
BCHO (%CHO)	49.0	46.7	47.1	1.85	47.3	47.9	1.78	0.18	0.59	0.98	0.15	0.23	48.2	0.64
BCHO (g/kg, DM)	250	239	241	14.2	243	244	13.9	0.19	0.84	0.97	0.17	0.22	248	0.55
EDCHO (%CHO)	51.0	53.3	52.9	1.85	52.7	52.1	1.78	0.18	0.59	0.98	0.15	0.23	51.8	0.64
EDCHO (g/kg, DM)	260	273	271	5.0	270	265	4.2	0.17	0.38	0.94	0.14	0.24	265	0.70

Notes: SEM: Standard error of means. Kd: the rate of degradation of D fraction (%/h); U: undegradable fraction; D: potentially degradable fraction; T0: lag time of digesta in the rumen; S: soluble fraction; BCHO: rumen bypass CHO; EDCHO: effectively degraded CHO. SEM: Standard error of means. L: Linear; Q: Quadratic. Means with different letters within the same row are different.

The samples conditioned for 50 sec tended to have a greater S but a lower D of CHO than those conditioned for 75 sec (19.3 vs. 17.3% CHO, 53.1 vs. 55.6% CHO, respectively; $P<0.10$). In addition, conditioning temperature tended to have a positive significant effect on S of CHO ($P<0.10$). Rumen bypass CHO (BCHO) and rumen effectively degraded CHO (EDCHO) were not affected by pelleting process under different conditioning temperatures and time ($P>0.05$).

3.3.3.3. *In situ* rumen degradation kinetics of NDF

Table 3.8 shows the effects of temperature and time of on rumen degradation kinetics of NDF of canola meal. The Kd and T0 of NDF in canola meal pellets were not affected by conditioning temperature and time ($P>0.05$).

The interaction of conditioning temperature and time had no significant effect on S of NDF ($P>0.05$). Conditioning time had a positive effect on S of NDF ($P<0.05$). The samples conditioned for 50 sec had a lower S of NDF than those conditioned for 75 sec (1.5 vs. 4.8% NDF). Meanwhile, conditioning temperature had a significant quadratic effect on S of NDF ($P=0.01$). Therefore, S of NDF was first increased, and then decreased with increasing conditioning temperature. The greatest S of NDF was found in the samples conditioned at 80ºC (5.9% NDF).

Conditioning temperature tended to have a significant impact on D of NDF ($P<0.10$), and the samples conditioned at 80ºC tended to have the lowest D of NDF (49.3% NDF) compared with those conditioned at 70ºC and 90ºC. The U of NDF in pellets was decreased with increasing conditioning time ($P<0.05$). However, U of NDF was not affected by conditioning temperature ($P>0.05$). Structural components such as cellulose, hemicellulose, and lignin are usually measured in NDF analysis (Van Soest et al., 1991). Hemicellulose consists of different sugar monomers and is much easier to be hydrolyzed than cellulose (Pasangulapati et al., 2012). Pasangulapati et al. (2012) summarized that hemicellulose can bind with cellulose and lignin, and serve as a linkage between cellulose and lignin. The possible changes in the linkage between lignin and hemicellulose, or between hemicellulose and cellulose during pelleting need to be investigated.

Table 3.8 Effects of temperature and time of conditioning during the pelleting process on *in situ* rumen degradation characteristics of neutral detergent fiber in canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
In situ rumen degradation														
K_d (%/h)	7.5	10.0	7.3	1.07	8.8	7.8	0.87	0.17	0.41	0.74	0.92	0.06	7.0	0.39
T_0 (h)	1.3	1.1	1.0	0.40	1.1	1.2	0.37	0.65	0.92	0.72	0.37	0.93	2.0	0.09
S (%NDF)	1.7[b]	5.9[a]	1.9[b]	1.39	1.5	4.8	1.20	0.05	0.03	0.35	0.91	0.01	1.3	0.29
D (%NDF)	53.2	49.3	53.8	2.22	52.2	52.0	2.08	0.06	0.91	0.67	0.77	0.02	54.4	0.25
U (%NDF)	45.1	44.8	44.3	3.01	46.3	43.2	2.95	0.86	0.01	0.44	0.60	0.90	44.1	0.65
BNDF(%NDF)	69.0[a]	65.5[b]	68.6[a]	1.76	68.4	66.2	1.67	0.01	0.05	0.66	0.77	<0.01	69.7	0.15
BNDF(g/kg, DM)	210	202	206	12.1	204	208	12.0	0.15	0.24	0.30	0.28	0.11	198	0.12
EDNDF(%NDF)	31.0[b]	35.5[a]	31.4[b]	1.8	31.4	33.8	1.67	<0.01	0.05	0.66	0.77	<0.01	30.6	0.15
EDNDF(g/kg,DM)	94[b]	112[a]	94[b]	3.8	93	107	3.1	<0.01	<0.01	0.20	0.97	<0.01	86	0.02

Notes: K_d: the rate of degradation of D fraction (%/h); U: undegradable fraction; D: potentially degradable fraction; T0: lag time of digesta in the rumen; S: soluble fraction in the *in situ* incubation; BDNDF: rumen bypass neutral detergent fiber; EDNDF: effectively degraded neutral detergent fiber. SEM: Standard error of mean; Means with different letters within the same row are significantly different; L: Linear; Q: Quadratic.

The interaction of conditioning temperature and time on rumen bypass NDF (BNDF) and rumen effectively degraded NDF (EDNDF) was not significant (P>0.05). Conditioning temperature had a significant quadratic effect on BDNDF and EDNDF percentages (P<0.01). The samples conditioned at 80ºC were lowest in BDNF (65.5% NDF) and highest in EDNDF (35.5% NDF). On a DM basis, EDNDF content was affected by both conditioning time and temperature (P<0.01), and the quadratic effect of the conditioning temperature was proven significant (P<0.01). The EDNDF content was increased with increasing conditioning time (93 vs. 107 g/kg DM). The samples conditioned at 80ºC had the highest EDNDF content (111 g/kg DM) compared with those conditioned at 70ºC and 90ºC. Pelleted samples were significantly higher in EDNDF (g/kg DM) than the unprocessed mash (P<0.05), suggesting that that more NDF was degraded in the rumen and that rumen bypass NDF was reduced after pelleting. Theodoridou and Yu (2013c) reported that differences in S, D, Kd, T0, BNDF, and EDNDF of NDF between canola meal and canola presscake were not significant (P>0.05). However, canola meal tended to have lower D, T0, and EDNDF, but higher BNDF than canola presscake.

3.3.3.4. *In situ* rumen degradation kinetics of CP

Effects of temperature and time of conditioning during pelleting on rumen degradation characteristics of protein are presented in Table 3.9. The interaction effect of conditioning temperature and time on Kd was not significant (P>0.05). However, conditioning temperature affected Kd of CP in canola meal pellets significantly (P<0.05), and its quadratic effect was proven significant (P<0.05). Therefore, Kd of CP was first increased, and then decreased with increasing conditioning temperature. Samples conditioned at 80ºC had the highest Kd at 10.7%/h. It was observed that the unprocessed mash had a lower Kd than pelleted samples (P<0.01), indicating that Kd of CP was increased after pelleting. Different processing methods have different effects on Kd of CP. Mustafa et al. (2003a) indicated that Kd of CP in flaxseed was decreased by extrusion at 155ºC with a conditioning time of 43 sec. Doiron et al. (2009) observed Kd of CP in flaxseed was decreased by increasing processing time of autoclaving (120ºC; 10, 20, and 30 min). Theodoridou and Yu (2013c) found that S of CP in canola presscake was higher than that in canola meal, indicating that desolventizing-toasting reduced S of CP.

Table 3.9 Effects of temperature and time of conditioning during pelleting process on *in situ* rumen degradation kinetics of crude protein in canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
In situ rumen CP degradation														
K_d (%h)	9.4^{ab}	10.7^{a}	8.9^{b}	0.47	9.8	9.5	0.39	0.03	0.58	0.70	0.48	0.01	7.8	<0.01
T_0 (h)	0.0	0.2	0.0	0.10	0.1	0.1	0.10	0.16	0.81	0.94	1.00	0.06	0.0	0.48
S (%CP)	11.5^{a}	10.2^{ab}	7.9^{b}	1.25	8.7	11.0	1.18	<0.01	0.01	0.78	<0.01	0.54	5.7	<0.01
D (%CP)	81.2^{b}	82.0^{ab}	85.2^{a}	1.92	83.2	82.5	1.84	0.02	0.52	0.92	<0.01	0.31	88.7	<0.001
U (%CP)	7.3	7.8	6.9	0.75	8.1	6.6	0.68	0.54	0.02	0.67	0.60	0.33	5.7	0.06
BCP (%CP)	39.7^{ab}	37.6^{b}	41.3^{a}	0.77	40.1	38.6	0.63	0.01	0.11	0.76	0.06	0.01	44.2	<0.001
RUP^{NRC} (g/Kg, DM)	157^{ab}	150^{b}	164^{a}	5.3	160	154	5.1	<0.01	0.13	0.79	0.08	<0.01	176	<0.001
EDCP (%CP)	60.9^{ab}	62.4^{a}	58.6^{b}	0.77	59.9	61.5	0.63	0.01	0.11	0.76	0.06	0.01	55.8	<0.001
EDCP (g/Kg, DM)	244^{ab}	249^{a}	234^{b}	7.5	239	246	7.1	<0.05	0.11	0.80	0.09	0.06	225	<0.01

Notes: K_d: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T_0: lag time of digesta in the rumen; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein. SEM: Standard error of means; L: Linear; Q: Quadratic; Means with different letters in the same row are significantly different.

The interaction effect of conditioning temperature and time on S of CP was not significant (P>0.05). Conditioning temperature had a negative effect on S of CP (P<0.01). Therefore, increasing temperature in the conditioner would decrease S of CP. Conditioning time exhibited a positive effect on S of CP: the samples conditioned for 75 sec had a higher S of CP than those conditioned for 50 sec (11.0 vs. 8.7% CP; P<0.05). The pellets had a higher S of CP than unprocessed mash (P<0.01), suggesting that pelleting improved S of CP in canola meal. Sadeghi and Shawrang (2006) observed S of CP reduced after microwave irradiation. In Doiron et al. (2009), S of CP in flaxseed decreased after autoclaving at 120ºC. Mustafa et al. (2003b) indicated that increasing processing time during autoclaving at 127ºC reduced S of CP in sunflower seed. A contrary result was observed when sunflower seed was extruded at 155ºC with a resident time of 43 sec: the S of CP increased (Mustafa et al., 2003a). Plaisance et al. (1997) reported that S of CP in canola meal decreased when roasted at 105ºC for 105 min. Goelema (1999) reported that S of CP in broken legume seed mixtures was increased after conditioning at 90ºC for 35 sec.

The interaction of conditioning temperature and time on D of CP was not significant (P>0.05). The D of CP was increased with increasing conditioning temperature (P<0.05). Compared with the unprocessed mash, pelleted samples exhibited a higher S (P<0.01) but a lower D (P<0.01) than the unprocessed mash, indicating pelleting process increased S but reduced D of protein. The interaction effect of conditioning temperature and time on U of CP was not significant (P>0.05). However, U of CP was negatively affected by the conditioning time (P<0.05). Samples conditioned for 75 sec was lower in U than those conditioned for 50 sec (6.5 vs. 8.1% CP). Average values of U of CP in pellets tended to be higher than that of unprocessed mash (P<0.10).

The effect of interaction of conditioning temperature and time on rumen bypass and effectively degraded fractions of CP was not significant (P>0.05). However, conditioning temperature had significant quadratic effects on BCP (%CP) (P=0.01) and EDCP (%CP) (P=0.01). The BCP was first decreased, and then increased with increasing conditioning temperature. In contrast, EDCP was first increased, and then decreased with increasing conditioning temperature. The samples conditioned at 80ºC were lowest in BCP (37.6% CP) but highest in EDCP (62.4% CP) in the current study. On a DM basis, the samples conditioned at 80ºC had the lowest RUP^{NRC} (150 g/kg DM) in comparison with those conditioned at 70ºC and 90ºC.

It was observed that the unprocessed mash had a lower effectively rumen degraded fraction but a higher rumen bypass fraction of CP than pellets ($P<0.01$). Therefore, pelleting shifted protein digestion to the rumen rather than to the small intestine. McKinnon et al. (1995) reported that the rumen degradation of CP was reduced when canola meal was dry-heated at different conditions (125 and 145ºC; 10, 20, and 30 min). Sadeghi and Shawrang (2006) indicated that microwave irradiation increased S and D of CP but reduced U of CP in canola meal. Also in that study, ED fraction of CP decreased with microwave irradiation, and radiation time exhibited a cubic effect on ED of CP. Plaisance et al. (1997) observed decreased S and D but increased Kd of CP after roasting at 150ºC for 105 min. It was reported that the disappearance of CP in the rumen was decreased by moist heating at 127ºC with increasing processing time (15, 30, 45, 60, and 90 min) (Nia and Ingalls, 1992). It should be noted that processing conditions used by these researchers were associated with relatively higher temperature and longer processing time, while processing conditions in the current study were associated with lower temperature and shorter processing time.

The non-covalent interactions involved in the protein structure, which correlate with protein inherent structure, could be affected by changing protein environmental temperature (Goelema, 1999). The coiled or pleated structure of protein can be uncoiled or unfolded during heat processing. In addition, heat processing can divide protein into its subunits, which can then be either unfolded or uncoiled (Goelema, 1999). In the current study, rumen degradation of CP of canola meal pellets was decreased with increasing conditioning temperature. However, unprocessed canola meal exhibited lower protein rumen degradation than pellets. A similar result was observed in Goelema (1999) when the mixture of broken legume seeds was pelleted after conditioning at 90ºC for 35 sec. Goelema (1999) found that increased protein degradability was associated with decreased particle size of the treatment samples after pelleting. Goelema (1999) also noted that changes in protein structure during the pelleting process should be taken into consideration and need to be investigated. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) was used to investigate these changes and clarify how they affect rumen degradation of CP.

3.3.4. Effects of temperature and time of conditioning during the pelleting process on hourly effective rumen degradability ratios and potential N-to-energy synchronization in pelleting processed canola meal

For dairy cattle, the optimal ratio between effective degradability of N and energy to achieve maximum microbial synthesis and minimum N loss is 32 g N/kg CHO or 25g N/kg OM (Sinclair et al., 1993). A potential loss of N or a deficiency in energy supply would be caused in the rumen when the ratio is higher than 25g N/kg or 32 g N/kg CHO. The N shortage for microbial growth is exhibited when the ratio is below optimal value (Nuez-Ortín and Yu, 2010).

Effects of conditioning temperature and time on degradability ratios and hourly effective degradability ratios of canola meal are shown in Table 3.10. Conditioning temperature exhibited significant effects on the ratio of effectively degraded N to effectively degraded CHO (ED_N/ED_CHO) and the ratio of effectively degraded N to effectively degraded OM (ED_N/ED_OM) of canola meal pellets ($P<0.10$). ED_N/ED_CHO and ED_N/ED_OM of canola meal pellets tended to be decreased with increasing conditioning temperature, indicting potential N loss of canola meal pellets in the rumen tended to decrease with increasing temperature. However, pellets had higher average values of ED_N/ED_CHO and ED_N/ED_OM than unprocessed mash ($P<0.05$), indicating that ED_N/ED_CHO and ED_N/ED_OM of canola meal were increased after pelleting in the current study.

The hourly ED_N/ED_CHO increased rapidly from 0 to 2 h for all samples. The hourly ED_N/ED_CHO at 0 h for pellets was higher than that of the unprocessed mash ($P<0.05$). Yu et al. (2008) indicated that the difference in hourly degradation of N was a main reason to change the hourly ED_N/ED_CHO ratio. Thus, the difference between unprocessed mash and pelleted samples in hourly ED_N/ED_CHO was likely due to the increased CP degradation caused by pelleting, which increased S and Kd of CP but decreased D of CP.

Table 3.10 Effects of temperature and time of conditioning during pelleting process on degradability ratios between N and CHO, N and OM, and hourly effective degradability ratios of canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
N to OM and N to CHO ratios														
N/OM	69.11	69.01	68.74	1.973	68.67	69.24	1.966	0.63	0.10	0.93	0.36	0.82	68.82	0.82
N/CHO	125.74	124.87	124.74	6.259	124.19	126.04	6.234	0.73	0.11	0.81	0.47	0.76	124.24	0.72
ED_N/ED_OM	76.52	74.91	72.75	1.204	73.72	75.74	1.051	0.06	0.10	0.84	0.02	0.83	71.25	0.03
ED_N/ED_CHO	147.88	144.26	136.40	3.611	138.96	146.74	3.019	0.08	0.07	0.64	0.03	0.62	126.17	<0.01
Hourly effective degradability ratios of N_CHO at individual time														
0h	96.08^{a}	68.76^{ab}	48.65^{b}	15.846	54.38	87.95	14.236	0.04	0.03	0.49	0.01	0.81	30.47	0.03
2h	170.13	175.73	178.93	9.829	184.21	165.55	8.782	0.72	0.05	0.09	0.43	0.89	161.77	0.25
4h	177.93	184.27	189.35	9.290	191.52	176.18	8.645	0.41	0.04	0.09	0.19	0.93	176.90	0.49
8h	192.38^{b}	199.83^{ab}	207.80^{a}	13.375	204.94	195.07	13.228	0.02	0.02	0.26	<0.01	0.95	203.81	0.50
12h	203.95^{b}	212.50^{ab}	221.52^{a}	18.065	216.07	209.25	17.892	0.04	0.19	0.58	0.01	0.97	226.83	0.10
24h	214.86	229.88	232.24	21.927	232.75	218.57	20.410	0.64	0.39	0.29	0.39	0.71	260.53	0.13
Hourly effective degradability ratios of N to OM at individual time														
0h	59.70^{a}	49.84^{ab}	37.68^{b}	5.601	40.58	57.57	4.643	0.03	0.01	0.59	0.01	0.86	28.36	0.02
2h	84.15	83.00	86.13	1.964	86.53	82.23	1.665	0.48	0.06	0.21	0.45	0.35	85.53	0.68
4h	83.61	83.26	86.03	1.582	86.26	82.34	1.356	0.34	0.03	0.21	0.24	0.38	85.87	0.45
8h	85.64^{b}	83.82^{ab}	85.85^{a}	1.585	85.81	82.40	1.502	0.05	<0.01	0.36	0.02	0.70	86.24	0.13
12h	81.82	84.43	85.69	2.004	85.50	82.47	1.886	0.09	0.04	0.95	0.03	0.65	86.40	0.19
24h	80.15	86.60	85.32	4.259	85.26	82.78	3.782	0.39	0.54	0.59	0.30	0.37	86.39	0.63

Notes: SEM: Standard error of means. ED: effective degradability; OM: organic matter; CHO: carbohydrates. L: Linear; Q: Quadratic. ratio unit for N/OM and ED_N/ED_OM: g N/kg OM; ratio unit for N/CHO and ED_N/ED_CHO: g N/kg CHO. Means with different letters within the same row are significantly different.

During the incubation period of 2 to 24 h, the samples processed under different conditions exhibited different hourly ED_N/ED_CHO ratio trends. Hourly ED_N/ED_OM ratio of the unprocessed mash kept increasing from 0 to 24 h. Meanwhile, pellets exhibited different hourly ED_N/ED_OM ratio trends.

Conditioning time exhibited significant effects on hourly ED_N/ED_CHO at 0, 4 and 8 h of incubation ($P<0.05$). The hourly ED_N/ED_CHO ratio of pellets increased with increasing conditioning time at 0 h of incubation, but decreased with increasing conditioning time at 4 and 8 h of incubation. Conditioning temperature showed significant impacts on hourly ED_N/ED_CHO ratios at incubation time of 0, 8 and 12 h ($P<0.05$). The hourly ED_N/ED_CHO ratios for pellets were decreased at 0 h but increased at 8 and 12 h with increasing conditioning temperature. Similar results were observed in hourly ED_N/ED_OM ratio; the ratio in all samples increased rapidly from 0 to 2 h of incubation time. The hourly ED_N/ED_OM ratios was increased at 0 h but decreased at 4, 8, and 12 h with increasing conditioning time ($P<0.05$). The hourly ED_N/ED_OM ratio of pellets at the incubation time of 0 h was positively affected by conditioning temperature ($P<0.05$).

Ratios of ED_N/ED_CHO and ED_N/ED_OM were higher than the optimal ratios. This indicated that there was a potential N loss or energy shortage in the rumen. In fact, canola meal is considered a protein source, while its energy content is limited. Increasing conditioning temperature could decrease potential N loss of canola meal pellets through reducing protein degradation of canola meal pellets in the rumen. Compared with optimal values, pelleting in our study increased rumen degradation of CP, which meant enzymatic degradation of protein can provide more N in the rumen for microbial synthesis. However, some of N did not get absorbed and used in microbial protein synthesis. The extra N means potential N loss, which will increase ammonia content in the rumen. The extra ammonia needs to be absorbed into the bloodstream, converted to urea in the liver, and excreted in the urine (Nuez-Ortín and Yu, 2010). With respect to the inclusion level of canola meal in diets, sufficient energy supply should be assured.

3.3.5. Effects of temperature and time of conditioning during the pelleting process on intestinal digestive characteristics of nutrients of canola meal

Effects of conditioning temperature and time on intestinal digestion of nutrients are presented in Table 3.11. Conditioning temperature tended to have a significant effect on intestinal digestible protein (IDP); samples conditioned at 90ºC tended to have highest IDP content than those conditioned at 70ºC or 80ºC. This was supported by the *in situ* experiment that CP degradation of canola meal pellets was lowest at conditioning temperature of 90ºC. The unprocessed mash tended to have a higher digestibility of intestinal digestible protein (dIDP) than pellets ($P<0.10$). The unprocessed mash was higher in IDP than pelleted samples ($P<0.01$). This was likely due to increased protein degradation in the rumen and reduced rumen bypass protein to the small intestine after pelleting. However, the total digestible protein (TDP) content was similar among samples, indicating that pelleting under different conditioning temperatures and time did not change TDP. Altering conditioning temperature and time did not affect CHO intestinal digestion. Intestinal digestibility of NDF was not changed by the pelleting process. However, the content of total digestible NDF (TDNDF) content was positively affected by conditioning time ($P<0.01$). The samples conditioned for 75 sec were higher in TDNDF than those conditioned for 50 sec on a DM basis (170 vs. 150 g/kg DM) and percentage basis (54.2 vs. 50.6% NDF). The TDNDF content (g/kg, DM) in the pellets was higher than that of the unprocessed mash ($P=0.01$).

3.3.6. Effects of temperature and time of conditioning during the pelleting process on predicted nutrient supply of canola meal to dairy cows based on the NRC 2001 model

The protein supply predicted using the NRC 2001 model is shown in Table 3.12. The interaction of conditioning temperature and time had no significant effect on predicted protein supply based on the NRC 2001 model ($P>0.05$).

Table 3.11 Effects of temperature and time of conditioning during the pelleting process on intestinal digestion and availability of CP, CHO and NDF in canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
CP intestinal digestion														
dIDP(%)	61.3	59.2	60.5	1.33	60.9	59.7	1.09	0.55	0.44	0.60	0.70	0.32	64.2	0.06
IDP(%CP)	24.0	22.3	25.0	0.83	24.5	23.1	0.68	0.09	0.16	0.66	0.41	0.04	28.4	<0.001
IDP(g/kg,DM)	96	89	100	4.5	97	92	4.1	<0.10	0.20	0.69	0.49	0.04	113	<0.01
TDP(%CP)	84.9	84.7	83.7	0.49	84.4	84.5	0.40	0.21	0.84	0.67	0.10	0.56	84.2	0.73
TDP(g/kg,DM)	340	338	334	10.6	336	339	10.5	0.29	0.44	0.86	0.12	0.85	336	0.79
CHO intestinal digestion														
dBCHO(%BCHO)	40.7	38.8	40.7	1.96	39.0	41.1	1.82	0.45	0.16	0.79	0.97	0.22	43.0	0.12
IDBCHO(%CHO)	20.0	18.1	19.2	0.87	18.5	19.7	0.71	0.35	0.27	0.86	0.53	0.19	20.7	0.20
IDBCHO(g/kg DM)	51	44	46	3.6	45	48	3.1	0.28	0.46	0.91	0.32	0.20	52	0.26
TDCHO(%CHO)	71.0	71.4	72.1	1.80	71.2	71.8	1.79	0.10	0.13	0.65	0.04	0.78	72.6	0.06
TDCHO(g/kg,DM)	311	316	317	2.21	316	313	1.8	0.14	0.39	0.90	0.07	0.41	317	0.39
NDF intestinal digestion														
dBNDF(%BNDF)	29.4	28.4	30.3	2.33	28.0	30.8	2.22	0.54	0.07	0.67	0.60	0.34	29.0	0.85
IDBNDF(%BNDF)	20.3	18.3	20.7	1.23	19.3	20.3	1.10	0.19	0.33	0.79	0.76	0.07	20.1	0.84
IDBNDF(g/kg,DM)	43	37	43	2.3	39	42	1.9	0.16	0.28	0.77	0.97	0.06	40	0.82
TDNDF(%NDF)	51.3	53.8	52.1	2.60	50.6	54.2	2.56	0.13	<0.01	0.41	0.48	0.06	50.6	0.19
TDNDF(g/kg, DM)	156	169	156	4.2	150	170	3.5	0.05	<0.001	0.09	0.90	0.02	144	0.01

Notes: SEM: Standard error of means. IDP: intestinal digestible protein, calculated as: BCP × dIDP; dIDP: intestinal digestibility of rumen undegraded protein; TDP: total digestible protein, calculated as: EDCP +IDP; BCHO: rumen bypassed carbohydrates; dBCHO: intestinal digestibility of rumen bypassed CHO, calculated as: (BCHO-CHO residual after 48h rumen incubation) / BCHO × 100; IDBCHO: intestinal digestible rumen bypassed CHO, calculated as: BCHO × dBCHO; TDCHO: total digestible CHO, calculated as: IDBCHO + EDCHO; dBNDF: intestinal digestibility of rumen bypassed NDF, calculated as:(BNDF-NDF residual after 48h rumen incubation) / BNDF × 100; IDBNDF: intestinal digestible rumen bypassed NDF, calculated as: BNDF × dBNDF; TDNDF: total digestible NDF, calculated as: EDNDF + IDBNDF. L: Linear; Q: Quadratic. Means with different letters within the same row are different.

Table 3.12 Effects of temperature and time of conditioning during the pelleting process on potential protein supply to dairy cows predicted by the NRC 2001 model for canola meal

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec	75 sec	SEM	Temp (n=6)	Time (n=6)	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
Truly absorbed rumen-synthesized microbial protein in small intestine														
MCP_{TDN} (g/kg, DM)	82.1	82.0	82.1	0.37	82.0	82.1	0.39	0.96	0.82	0.11	0.98	0.78	82.3	0.24
MCP^{NRC} (g/kg, DM)	82.1	82.0	82.1	0.37	82.0	82.1	0.39	0.96	0.82	0.11	0.98	0.78	82.3	0.24
$AMCP^{NRC}$ (g/kg, DM)	52.5	52.5	52.5	0.24	52.5	52.5	0.24	0.96	0.84	0.10	1.00	0.78	52.6	0.26
Truly absorbed rumen-undegraded feed protein in small intestine														
RUP^{NRC} (g/kg, DM)	156.7^{ab}	149.9^{b}	164.3^{a}	5.34	159.6	154.3	5.08	<0.01	0.13	0.79	0.08	<0.01	176.3	<0.001
$ARUP^{NRC}$ (g/kg, DM)	98.9	90.0	99.5	4.52	97.4	92.3	4.10	<0.10	0.20	0.69	0.49	0.04	113.3	<0.01
Truly digested rumen endogenous protein in small intestine														
ECP(g/kg, DM)	10.3	10.4	10.5	0.07	10.6	10.2	0.06	0.06	<0.001	0.55	0.02	0.62	11.1	<0.0001
$AECP^{NRC}$ (g/kg, DM)	4.1	4.1	4.2	0.03	4.2	4.1	0.02	0.05	<0.001	0.58	0.02	0.56	4.4	<0.0001
Total truly absorbed protein in small intestine														
MP^{NRC} (g/kg, DM)	152.8	145.5	156.2	4.71	154.1	148.9	4.30	0.09	0.19	0.70	0.48	0.04	170.4	0.001
Degraded protein balance														
OEB^{NRC} (g/kg, DM)	147.4	152.1	137.5	7.10	141.9	149.5	6.72	0.05	0.11	0.83	0.09	0.06	125.1	<0.01

Notes: SEM: Standard error of means. MCP_{TDN}: microbial protein synthesized in the rumen based on discounted TDN; MCP^{NRC}: microbial protein; $AMCP^{NRC}$: truly absorbed microbial protein in the small intestine; RUP^{NRC}: rumen undegradable feed crude protein; $ARUP^{NRC}$: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; $AECP^{NRC}$: truly absorbed rumen endogenous protein in the small intestine; MP^{NRC}: metabolizable protein; OEB^{NRC}: degraded protein balance in the NRC 2001 model. L: Linear; Q: Quadratic. Means with different letters within the same row are different

Microbial protein synthesized in the rumen based on discounted TDN (MCP^{TDN}) and truly absorbed microbial protein in the small intestine (AMCP), were not significantly different among the samples (P>0.05). This indicates pelleting under different conditioning temperatures and time did not affect microbial protein production in the rumen based on available energy of canola meal. After pelleting with relative low conditioning temperature in this study, protein degradation of canola meal was increased in the rumen to provide more N for microbial synthesis. However, in the current study, the energy level of canola meal became the restriction for improving microbial protein synthesis. Therefore, microbial protein production based on the NRC 2001 model was not changed. Conditioning temperature tended to have a significant effect on truly absorbed rumen undegradable protein in the small intestine ($ARUP^{NRC}$) (P<0.10), and the samples conditioned at 80ºC tended to have the lowest $ARUP^{NRC}$ compared with those conditioned at 70ºC and 90ºC. Rumen endogenous protein (ECP) was negatively affected by conditioning time (P<0.01). The samples conditioned for 50 sec had a higher ECP content than those conditioned for 75 sec (10.6 vs. 10.2 g/kg DM). As a consequence, truly absorbed rumen endogenous protein in the small intestine (AECP) in the samples conditioned for 50 sec was higher than that in those conditioned for 75 sec (4.2 vs. 4.1 g/kg DM; P<0.01). Conditioning temperature tended to have a positive effect on metabolizable protein (MP^{NRC}) content. MP^{NRC} content of canola meal pellets tended to be increased with increasing temperature. Samples conditioned at 90ºC had highest MP^{NRC} value than those conditioned at 70ºC or 80ºC. Conditioning temperature tended to have a significant effect on OEB^{NRC}, and numerically the lowest value of 137.5 g/kg DM was observed in the samples conditioned at 90ºC (P<0.10). Therefore, increasing conditioning temperature tended to increase protein supply and reduce protein N loss of canola meal pellets. Compared with pellets, the unprocessed mash exhibited significant differences in RUP^{NRC}, $ARUP^{NRC}$, ECP, $AECP^{NRC}$, MP^{NRC}, and OEB^{NRC} (P<0.01). The unprocessed mash was higher in RUP^{NRC}, $ARUP^{NRC}$ and MP^{NRC} but lower in ECP, $AECP^{NRC}$, and OEB^{NRC}. This was likely due to increased protein degradation of canola meal in the rumen after pelleting, which provided less bypass protein to the small intestine. However, microbial protein synthesis based on energy supply was not increased, despite the extra N in the rumen.

3.3.7. Effects of temperature and time of conditioning during the pelleting process on feed milk values (FMV) of canola meal

The result of predicted feed milk values (FMV) based on the NRC 2001 model is presented in Table 3.14. No significant interaction effect of conditioning temperature and time on FMV of canola meal pellets was observed ($P>0.05$). Conditioning temperature tended to have a positive effect on FMV of canola meal pellets ($P<0.10$). Samples conditioned at 90ºC had the highest FMV, 3.2 kg milk/kg feed, compared with those conditioned at 70ºC or 90ºC. The FMV was significantly different between unprocessed mash and pellets ($P<0.05$). The FMV of the unprocessed mash was higher than that of pellets. Therefore, increasing conditioning temperature tended to increasing FMV within pelleting treatments. However, it was revealed that the pelleting process with relative low temperature and short feed processing time in the current study might reduce the FMV of canola meal.

3.4. Conclusion

In conclusion, the pelleting process in the current study with different conditioning temperatures (70, 80 and 90ºC) and time (50 and 75 sec) can affect nutrient profiles, degradation and digestion characteristics, and predicted protein supply of canola meal pellets. In contrast to processing with high temperatures and long time periods, conditions in our study were associated with low temperatures and short time periods. Changes in temperature and time during the conditioning stage altered protein profiles. The SCP was decreased and the NDICP was increased with increasing conditioning time. However, CHO profiles and predicted energy profiles were not manipulated. The rumen degradation kinetics of protein were affected by conditioning temperature and time. Meanwhile, the CP degradation increased in pelleting with low temperatures, and the protein digestion site was shifted to the rumen rather than to the small intestine. When the conditioning temperature was set to 90ºC, rumen degradation of nutrients such as CP and NDF of canola meal pellets were lowest, while the highest post-rumen digestion of them was observed. Available protein for intestinal digestion of canola meal pellets became more because of increased rumen bypass protein to the small intestine. The pelleting process tended to affect the intestinal digestibility of canola meal protein. However, the total digestible CP content was not affected.

Table 3.13 Effects of temperature and time of conditioning during pelleting process on feed milk value (FMV) of canola meal, based on metabolic characteristics of protein, predicted using the NRC 2001 model

Items (kg milk per kg feed)	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Control (Raw) (n=2)	Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time		Control vs. processed
Feed milk value (NRC 2001 model)	3.1	3.0	3.2	0.10	3.1	3.0	0.09	0.09	0.19	0.70	3.5	0.001

Notes: SEM: Standard error of means. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (NRC, 2001) and protein composition in milk is assumed to be 33 g protein per 1000 g milk.

With regard to the predicted protein supply of canola meal based on the NRC 2001 model, MP value of canola meal pellets tended to increase with increasing conditioning temperature. However, the MP value was decreased after pelleting process with low temperature in this study. A reduced MP value meant reduced truly absorbed protein content in the small intestine and reduced protein supply to dairy cattle. In addition, the OEB^{NRC} value tended to be lowest when conditioning temperature was set at 90ºC because of the lowest protein degradation of pellets occurred at 90ºC. In addition, pelleting in the current study intensified potential N loss of canola meal in the rumen due to the increased OEB^{NRC} value.

Therefore, this study demonstrated that the pelleting process changes nutrient profiles, rumen degradation and intestinal digestion characteristics, and the predicted protein supply of canola meal. However, pelleting-induced inherent structural changes in relation to nutrient values and availability of canola meal remained undetermined. Thus, it is worth revealing inherent structural changes induced by the pelleting process, and determine the relationship between inherent molecular structure and nutrient profiles, rumen degradation and intestinal digestion characteristics, as well as the predicted protein supply of canola meal.

4. Detecting Pelleting-Induced Changes in Inherent Structures of Canola Meal under Different Processing Conditions by Using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) Molecular Spectroscopy

4.1. Introduction

For maintaining accuracy in feed formulation, it is necessary to reveal the effects of feed processing on chemical composition, nutrient digestion characteristics, and predicted nutrient supply on a bio-molecular basis. However, conventional chemical analysis can only reveal how processing affects the chemical composition of feed. Fully understanding the effect of temperature and time of conditioning during pelleting on nutrient profiles, digestion characteristics, and predicted protein supply of canola meal not only relies on information about chemical changes but also information about inherent structural changes. Recently, mid-IR molecular spectroscopy has been applied in feed analysis to explain and map feed structures and quantify inherent structure of proteins (Yu, 2004; Yu et al., 2004). ATR-FTIR is a kind of FTIR spectroscopy based on the method of total internal reflectance. It has been used recently in feed analysis for different purposes (Büchl et al., 2010; Yu and Nuez-Ortín, 2010; Mahesar et al., 2011; Gamage et al., 2012; Zhang and Yu, 2012a, b; Abeysekara et al., 2012, 2013; Belanche et al., 2013; Nietner et al., 2015). The ATR-FTIR exhibits several advantages. Sample preparations such as grinding, squishing and pressing are not required. In addition, ATR-FTIR can handle various kinds of samples, such as liquids, solids, powders, and polymers. ATR-FTIR not only provides a rapid and easy measurement of spectra, but is also not destructive to samples. Excellent signal to noise ratios (SNRs) provided by ATR-FTIR ensure precise measurements of spectra (Smith, 2011). Hence, it is worth using ATR-FTIR to investigate inherent structural changes induced by pelleting under different conditioning temperatures and time. The objectives of the current study were: 1) to detect the molecular structure of canola meal processed by pelleting, under different conditioning temperatures and time; 2) to identify differences in molecular structures among pellets processed under different conditioning temperatures and time, and between the unprocessed mash and pellets; and 3) to quantify inherent structural changes in relation to chemical composition, energy content, rumen degradation, intestinal digestion, and predicted protein supply. The hypothesis for this study was that processing-induced inherent structural changes affected nutrient profiles, digestion characteristics, and predicted protein supply of canola meal for dairy cattle.

4.2. Materials and Methods

4.2.1. Samples for ATR-FTIR

Pellets produced under different conditioning temperatures (70, 80 and 90ºC) and time (50 and 75 sec) and unprocessed mash were used in this study. All fourteen samples were ground through a 1 mm screen using a Retsch ZM 200 rotor mill. The FTIR analysis was performed at the molecular spectroscopy lab of the Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada using an AJASCO FT/IR-4200 (JASCO Corporation, Tokyo, Japan). The ATR-FTIR machine is equipped with a ceramic IR light source and a deuterated L-alanine doped triglycine sulfate detector that contains an MI Racle attenuated total reflectance (ATR) accessory module and outfitted with a ZnSe crystal and pressure clamp (PIKE Technologies, Madison, WI, USA). Spectra were collected at the mid-IR infrared region from ca. 4000-700 cm^{-1} with a spectral resolution of 4 cm^{-1} with 128 scans co-added. To minimize infrared absorption by CO_2 and water vapour in the ambient air, background spectra were collected with 256 scans. JASCO Spectra manager II software was used to collect spectra data. Spectral data of each sample was collected five times, and average values were used in study. OMNIC 7.3 (Thermo-Nicolet, Madison, WI, USA) software was used to identify and collect spectral information of functional groups of protein, cellulosic components, and carbohydrate. Absorption peak parameters such as center region, baseline region, height, and area were determined according to the literature (Xin and Yu, 2013a, b; Theodoridou and Yu, 2013a; Yang et al., 2013b; 2014)

4.2.2. Univariate analysis

Spectral bands of functional groups were detected based on the literature (Peng et al., 2014a, b; Theodoridou and Yu, 2013a; Xin and Yu, 2013a, b; Yang et al., 2013b). While identifying the secondary structure of protein, the baseline was set at ca. 1718-1481 cm^{-1}. Amide I consists of 80% C=O stretching vibrations and 20% C-N stretching vibrations; amide II consists of 60% N-H bending vibrations and 40% stretching vibrations (Jackson and Manstch, 1995, 2000; Yu, 2004).

Band absorption of amide I, amide II, α-helix, and β-sheet was determined based on previous studies (Jackson and Manstch, 1995, 2000; Yu, 2004; Xin and Yu, 2013b; Yang et al., 2013b). Amide I and amide II are two major bands associated with the protein secondary structure (Yu, 2004). However, amide I is the first band used when studying the protein

secondary structure, rather than amide II. This is because amide II is affected by multiple functional groups, which lead to complex vibrations, and, therefore, has a lower sensitivity when predicting protein secondary structure (Jackson and Mantsch, 2000). Amide I can be further divided into several multi-component peaks using the Second Derivative Function in OMNIC 7.3. The α-helix and β-sheet were then selected from these multi-components. The α-helix peak center was located at ca. 1660-1650 cm^{-1} and β-sheet peak center was located at ca. 1627-1618 cm^{-1}. These spectral bands of protein are shown in Appendix, Figure 8.1. In addition, ratios of amide I height to amide II height, amide I area to amide II area, and α-helix height to β-sheet height were calculated. Therefore, for amide I, peak center ranged at ca. 1644-1625 cm^{-1} and area ranged at ca. 1718-1567 cm^{-1}. For protein amide II, peak center ranged at ca. 1538-1535 cm^{-1} and area ranged at ca. 1567-1481 cm^{-1}.

Functional groups of carbohydrates, spectral bands of cellulosic compounds (CEL), structural CHO (SCHO), and total CHO (TCHO) were determined based on previous studies (Wetzel et al., 1998; Samadi and Yu, 2011; Xin and Yu, 2013a; Yang et al., 2013b). For cellulosic compounds, the baseline and the area both ranged from ca. 1302-1186 cm^{-1}. The height center range of cellulosic compounds was found at ca. 1234-1228 cm^{-1} (see Appendix, Figure 8.1). The baseline for structural CHO was located at ca.1488-1186 cm^{-1}, which was set as the same as its area range. Then, the heights of three major structural CHO peaks were detected at ca.1417-1409 cm^{-1}, 1393-1367 cm^{-1}, and 1235-1227 cm^{-1}, respectively. The area of structural CHO was recorded at ca. 1488-1186 cm^{-1} (see Appendix, Figure 8.1). Baseline and area regions ranged at ca. 1193-879 cm^{-1} for total CHO. The heights of three major total CHO peaks (1, 2, and 3) were detected at ca. 1159-1150 cm^{-1}, ca. 1107-1103 cm^{-1}, and ca. 1051-1029 cm^{-1}, respectively. Area regions for three peaks were located at ca. 1192-1126 cm^{-1}, ca. 1126-1086 cm^{-1}, and ca. 1086-881 cm^{-1}, respectively. Spectral data listed above are identified in Appendix, Figure 8.1 and recorded for each sample. SAS 9.3 was used to detect treatment differences in spectra data among the samples.

4.2.3. Multivariate analysis

Two different methods were employed in the current study to perform multivariate spectral analysis using Statistical 8.0 (StatSoft Inc., Tulsa, OK, USA). The Agglomerative Hierarchical Cluster Analysis (CLA), which uses Ward's Algorithm method without parameterization for clustering, presents results as dendrograms (Yu, 2005a, b, c; Yu, 2008). The Principal Component Analysis (PCA) transforms all interrelated variances into new

uncorrelated variances called principal components (PCs) (Yu, 2005a, b, c; Yu, 2008). The result of PCA is presented as a scatter plot using two main PCs, in the form of PC1 vs. PC2. The first few PCs would account for >95% of remaining observed variance. Detailed procedures of these two methods were reviewed in a series of papers (Yu, 2005a, b, c; Yu, 2008). Functional groups of protein (ca. 1718-1567 cm^{-1}), cellulosic compounds (ca. 1302-1186 cm^{-1}), structural CHO (ca. 1488-1186 cm^{-1}), and total CHO (ca. 1186-881 cm^{-1}) were analyzed using multivariate molecular spectral analysis.

4.2.4. Statistical analysis.

For univariate analysis, the experiments were designed using the randomized complete block design (RCBD) with a 3 × 2 factorial treatment arrangement. Statistical analyses were performed using the MIXED procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, USA) using the following model:

$$Y_{ijkrt} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijr},$$

in which, Y_{ijr} was an observation of the dependent variable *ijr*; μ was the population mean for variable; α_i was the effect of conditioning temperature; β_j was the effect of conditioning time; γ_k is the interaction of conditioning time and temperature; e_{ijkrt} was the random error associated with observation *ijr*.

A contrast statement was performed to detect the difference between the unprocessed mash (raw) and the pellets. In addition, polynomial contrasts (linear and quadratic) of conditioning temperature were analyzed when the linear effect of the conditioning temperature was significant. Significance level for all statistical analyses was declared at $P<0.05$ and trends at $P<0.10$. Treatment means were compared using Tukey-Kramer method.

4.3. Results and Discussion

4.3.1. Quantifying protein and CHO molecular structures of canola meal processed under different pelleting conditions using ATR-FTIR

4.3.1.1. Determining protein molecular spectral characteristics of canola meal at amide I and amide II regions using univariate analysis

Effects of conditioning temperature and time on protein and CHO spectral profiles are shown in Table 4.1. In the protein amide spectral region (ca. 1481-1718 cm^{-1}), the amide I height and area in canola meal pellets were not affected by conditioning temperature, time, or their interaction ($P>0.05$). In addition, the unprocessed mash was not significantly different from pellets in terms of amide I height and area ($P>0.05$). Conditioning time and conditioning temperature showed significant effects on amide II area ($P<0.05$), but the interaction was not significant ($P>0.05$). Samples conditioned at 70ºC had the highest amide II area among samples conditioned at different temperatures. Samples conditioned for 50 sec were lower in amide II area than those conditioned for 75 sec (1.92 vs. 2.10; $P<0.05$). These changes indicated that chemical bonds of N-H and C-N in protein peptide groups of canola meal pellets were more sensitive to changes of temperatures and time applied during the conditioning stage. The vibrations of these chemical bonds may be changed during the conditioning stage. Similarly, the interaction of conditioning temperature and time on amide II peak height was not significant ($P>0.05$). Conditioning temperature exhibited a negative effect on amide II height so that the samples conditioned at 70ºC were highest in amide II peak ($P<0.01$). Conditioning time had a positive effect on amide II height ($P<0.01$): the samples conditioned for 75 sec had a higher amide II peak height than those conditioned for 50 sec (0.041 vs. 0.036).

In IR spectroscopy, obtaining precise values of protein secondary structural profiles for every sample is impossible. However, spectra data from different samples are comparable if samples are processed in similar ways (Yu, 2006). In addition, the sensitivity of mid-IR spectroscopy will result in differences in spectra measurement due to differences in methodologies, selected regions and baselines among studies (Yu, 2006; Doiron et al., 2009).

Table 4.1 Effects of temperature and time of conditioning during the pelleting process on protein and carbohydrates spectral profiles of canola meal using ATR-FTIR

Items	Conditioning Temperature (Temp)				Conditioning Time (Time)			P value			Polynomial contrast for Temp (P value)			Contrast P value
	70ºC (n=4)	80ºC (n=4)	90ºC (n=4)	SEM	50 sec (n=6)	75 sec (n=6)	SEM	Temp	Time	Temp×Time	L	Q	Control (Raw) (n=2)	Control vs. processed
Protein molecular structure spectral profiles														
Amide I area	5.02	4.71	4.78	0.128	4.76	4.91	0.105	0.21	0.32	0.18	0.19	0.23	4.95	0.56
Amide II area	2.14[a]	1.95[b]	1.95[b]	0.060	1.92	2.10	0.048	0.04	0.01	0.44	0.03	0.18	1.87	0.13
Amide I peak height	0.059	0.055	0.056	0.0015	0.055	0.057	0.0013	0.15	0.29	0.22	0.21	0.13	0.057	0.72
Amide II peak height	0.041[a]	0.037[b]	0.037[b]	0.0012	0.036	0.041	0.0009	<0.01	<0.001	0.69	<0.01	0.17	0.036	0.26
α-helix height	0.056	0.052	0.054	0.0015	0.053	0.055	0.0012	0.19	0.19	0.30	0.30	0.13	0.054	0.85
β-sheet height	0.056	0.052	0.053	0.0014	0.053	0.054	0.0012	0.25	0.40	0.20	0.28	0.20	0.055	0.69
Ratio of Amide I to Amide II height	1.43[b]	1.49[b]	1.54[a]	0.024	1.56	1.41	0.020	0.01	<0.001	0.09	<0.01	0.83	1.57	0.02
Ratio of Amide I to Amide II area	2.35[b]	2.44[a]	2.45[a]	0.035	2.48	2.35	0.030	0.04	<0.001	0.17	0.02	0.29	2.67	<0.0001
Ratio of α-helix to β-sheet height	1.01	1.00	1.01	0.015	1.00	1.01	0.015	0.38	0.05	0.50	0.88	0.17	0.98	0.03
Total carbohydrate structure spectral profiles														
TCHO peak 1 height	0.025	0.021	0.022	0.0015	0.022	0.023	0.0013	0.23	0.66	0.74	0.19	0.27	0.024	0.69
TCHO peak 2 height	0.051	0.049	0.063	0.0067	0.059	0.049	0.0055	0.28	0.23	0.48	0.22	0.31	0.055	1.00
TCHO peak 3 height	0.085	0.079	0.082	0.0045	0.084	0.081	0.0043	0.17	0.30	0.10	0.38	0.098	0.093	0.01
TCHO peak 1 area	1.00	0.95	0.99	0.043	0.99	0.97	0.039	0.55	0.56	0.57	0.87	0.28	1.09	0.03
TCHO peak 2 area	1.89	1.77	1.86	0.087	1.86	1.81	0.081	0.29	0.46	0.19	0.75	0.13	1.98	0.10
TCHO peak 3 area	9.49	8.78	9.23	0.475	9.36	8.97	0.452	0.15	0.19	0.11	0.47	0.09	10.21	0.02
Total area	12.38	11.50	12.08	0.602	12.22	11.76	0.570	0.18	0.24	0.13	0.54	0.08	13.27	0.02
Structural carbohydrates structure spectral profiles														
SCHO peak 1 height	0.018	0.017	0.017	0.0010	0.017	0.018	0.0010	0.06	0.64	0.13	0.02	0.45	0.018	0.18
SCHO peak 2 height	0.012	0.012	0.012	0.0011	0.012	0.012	0.0011	0.37	0.13	0.09	0.17	0.73	0.014	<0.01
SCHO peak 3 height	0.013	0.012	0.013	0.0006	0.013	0.013	0.0005	0.15	0.42	0.03	0.51	0.07	0.014	<0.01
SCHO area	3.10	2.88	2.91	0.202	2.98	2.95	0.1975	0.10	0.71	0.20	0.09	0.20	3.22	0.04

Table 4.1 Cont'd.

Cellulosic compounds spectral profiles													
CEL height	0.011	0.010	0.011	0.0004	0.011	0.011	0.0004	0.15	0.31	0.051	0.80	0.06	0.013	<0.01
CEL area	0.60	0.57	0.60	0.022	0.56	0.58	0.0195	0.25	0.54	0.052	0.93	0.10	0.65	0.01

Notes: SEM: Standard error of means. TCHO: total carbohydrates; SCHO: structural carbohydrates; CC: cellulosic compounds; L: Linear; Q: Quadratic. The unit for spectral data is IR absorbance unit. Baseline for protein spectral peak: ca. 1481-1718 cm^{-1}; protein amide I region: ca. 1718-1567 cm^{-1}; protein amide II region: ca. 1567-1481 cm^{-1}; center range of amide I peak: ca. 1644-1625 cm^{-1}; center range of amide II peak: ca. 1538-1535 cm^{-1}; center range for α-helix: ca. 1660-1650 cm^{-1}; center range for β-sheet: ca. 1627-1618 cm^{-1}. Total carbohydrate baseline and region: ca.1193-879 cm^{-1}; total carbohydrate peak 1 region: ca. 1192-1126 cm^{-1}; total carbohydrate peak 1 center range: ca.1159-1150 cm^{-1}; total carbohydrate peak 2 region: ca. 1126-990 cm^{-1}; total carbohydrate peak 2 center range: ca. 1170-1103 cm^{-1}; total carbohydrate peak 3 region: ca. 990-881 cm^{-1}. Baseline for structural carbohydrates: ca. 1488-1186 cm^{-1}; structural carbohydrate peak1 height center range: ca. 1417-1409 cm^{-1}; structural carbohydrates peak 2 height center range: ca. 1393-1367 cm^{-1}; structural carbohydrate peak 3 height center range: ca. 1235-1227 cm^{-1}. Baseline and region for cellulosic compounds: 1302-1186 cm^{-1}; cellulosic height center range: ca. 1234-1228 cm^{-1}. Means with different letters within the same row are significantly different.

Biological component ratio can be calculated as the quotient between areas or heights from two different functional group bands at each pixel (Yu, 2005c). Application of biological ratio has advantages in spectral analysis. Spectral variations caused by different tissue thicknesses of samples can be avoided. Determining nutrient utilization and availability of feed based on biological ratios could be possible. This is because the ratios are correlated with contents of biological components (Yu, 2005c). The ratios of amide I height to amide II height and amide I area to amide II area were positively affected by conditioning temperature ($P<0.05$) but negatively affected by conditioning time ($P<0.001$). The interaction was not significant ($P>0.05$). The ratios of amide I height to amide II height and amide I area to amide II area was increased with increasing conditioning temperature and decreased with increasing conditioning temperature. Therefore, the samples conditioned at 90ºC had the highest ratios of amide I height to amide II height (2.54, $P<0.05$) and amide I area to amide II area (1.45, $P<0.05$) compared with those conditioned at 70ºC and 80ºC. Samples conditioned for 50 sec were higher than those conditioned for 75 sec in ratio of amide I height to amide II height (1.56 vs. 1.41) and ratio of amide I area to amide II area (2.48 vs. 2.35). Compared with the unprocessed mash, pellets exhibited higher values of these two ratios ($P<0.05$). The ratio of amide I to amide II can be significantly affected by feed materials, heat processing, and genetic modification (Liu et al., 2012). They also observed that changes in area ratio of amide I to amide II were associated with changes in protein utilization and availability of cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS). Theodoridou and Yu (2013b) observed canola meal had a lower amide I to amide II area ratio than canola presscake, indicating that the area ratio was reduced after desolventizing-toasting during canola processing. However, Samadi et al. (2013) indicated that no difference in the area ratio of amide I to amide II was observed when autoclaved (121ºC; 1 h) and roasted (120ºC; 1 h) canola seeds were compared with unprocessed canola seeds. No significant effect of conditioning temperature, time, or their interaction on α-helix height and β-sheet height was observed ($P>0.05$). The difference between the unprocessed mash and the pellets was not significant ($P>0.05$).

With respect to the ratio of α-helix to β-sheet, conditioning temperature, time, or their interaction did not exhibit a significant impact ($P>0.05$). However, the unprocessed mash was significantly different from pellets with a lower ratio of α-helix to β-sheet ($P<0.05$). Doiron et al. (2009) indicated that changes in nutrient values of feeds were associated with changes in the ratio of α-helix to β-sheet. Yu (2005) indicated that a lower protein value was associated

with the increased content of β-sheet structure, which reduces accessibility of gastrointestinal enzymes for protein. Yu et al. (2004) observed that raw feather with a high protein content of 84% had a low protein digestibility of 5%. This was partially because the high level of β-sheet was negatively correlated with protein digestibility. Theodoridou and Yu (2013a) found that canola meal had lower α-helix and β-sheet heights than canola presscake. In their study, α-helix height was positively correlated with ADICP, indicating that a higher α-helix height may result in a lower protein digestibility. They also found that the ratio of α-helix to β-sheet played an important role in determining ruminal and intestinal digestion of protein. Yu (2005a) indicated that an increase in β-sheet height after heat treatment was likely due to protein aggregation required to form intermolecular β-sheet structures. Yu (2005c) reported that reduced α-helix and increased β-sheet were observed in roasted golden flax seeds. In addition, the ratio of α-helix to β-sheet was decreased from 1.28 to 0.73. In Doiron et al. (2009), an increased ratio of α-helix to β-sheet was observed in the endosperm area of autoclaved flaxseed (120ºC; 20, 40, and 60 min). In addition, the height ratio of α-helix to β-sheet correlated with protein profiles and digestive characteristics. Samadi and Yu (2011) reported that autoclaving (120ºC; 1 h) reduced α-helix and β-sheet heights of soybean seeds; therefore, the ratio of α-helix to β-sheet in soybean seed was reduced. Samadi et al. (2013) observed there was no difference in α-helix and β-sheet heights in raw, autoclaved, and roasted canola seeds. However, the ratio of α-helix to β-sheet, which correlated with rumen degradation and intestinal digestion characteristics of protein, decreased with autoclaving at 121ºC for 60 min, and increased with roasting at 120ºC for 60 min. Peng et al. (2014b) reported the ratio of α-helix to β-sheet in camelina seeds reduced with autoclaving at 120ºC for 60 min, and this was associated with decreased rumen degradation but increased intestinal digestion of protein. In our study, the lower ratio of α-helix to β-sheet was observed in the unprocessed mash, which was in accordance with the higher rumen degradation and lower intestinal digestion of protein found in the pelleted samples. Again, a protein's structural stability is sensitive to protein environmental conditions. It should be kept in mind that the combination of processing conditions such as temperature, time, and pressure could have effects on the protein inherent structure.

4.3.1.2. Determining carbohydrate molecular spectral characteristics of canola meal at total carbohydrates (TCHO), structural carbohydrates (SCHO) and cellulosic compounds regions using univariate analysis

Structural carbohydrates (CHO) (SCHO) usually include cellulosic and hemicellulosic compounds, while starch and sugars are included in non-structural CHO. The CHO structural characteristics could affect nutrient profiles and rumen degradation and intestinal digestion characteristics of feeds (Yu et al., 2011; Yang et al., 2014). Peak heights of total carbohydrates (TCHO) peak 1 and peak 2 were not affected by the pelleting process under different conditioning temperatures and time ($P>0.05$). The unprocessed mash exhibited a higher peak height of TCHO peak 3 compared with the pellets ($P=0.01$), though altering conditioning temperature and time did not affect peak height of TCHO peak 3 in the pellets ($P>0.05$). With respect to peak area of TCHO peaks, the unprocessed mash had higher peak area values of TCHO peak 1 and peak 3 and total area of TCHO than pellets ($P<0.05$). The difference between the unprocessed mash and the pellets in peak areas of TCHO peak 2 was not significant ($P>0.05$). Hence, the pelleting process in our study reduced TCHO spectral profiles in terms of TCHO area, TCHO peak 3 height, TCHO peak 1 area, and TCHO peak 3 area, though temperatures and time of conditioning had no significant effect on these spectral profiles.

Interaction of conditioning temperature and time showed a significant effect on peak height of SCHO peak 3 ($P<0.05$), though effects of conditioning temperature and time were not significant ($P>0.05$). When conditioning time was 50 sec, the samples conditioned at 70ºC had a similar value of SCHO peak 3 height to those conditioned at 80ºC, but a higher value of SCHO peak 3 height than those conditioned at 90ºC. When conditioning time was 75 sec, the value of SCHO peak 3 height of the samples conditioned at 90ºC was similar to those conditioned at 70ºC, but higher than those conditioned at 80ºC (Figure 4.1). Peak height values of SCHO peak 2 and 3 in the unprocessed mash were significantly higher than pelleted samples ($P<0.01$). Also, pelleting decreased the value of total SCHO area ($P<0.05$).

Cellulosic compounds (CEL), associated with cell wall structure, contain phenolic-carbohydrate complexes, hemicellulose encrustation and cellulose crystallinity (Yang, 2012). Interaction of conditioning temperature and time tended to have a significant effect on CEL height ($P<0.10$) (Figure 4.2). When conditioning time was 50 sec, samples conditioned at 80ºC and 90 ºC were not different in CEL height, but samples conditioned at 70ºC tended to have higher CEL height than those conditioned at 80ºC or 90ºC. When conditioning time was 75 sec, samples conditioned at 70ºC and 80ºC were not different in CEL height, but samples conditioned at 90ºC tended to have higher CEL height than those conditioned at 70ºC or 80ºC. Interaction effect of conditioning temperature and time on CEL was also tended to be significant ($P<0.10$) (Figure 4.3). When conditioning time was 50 sec, samples conditioned at 70ºC tended to have higher CEL area than those conditioned at 80ºC or 90ºC. When conditioning time was 75 sec, samples conditioned at 90ºC tended to have higher CEL area than those conditioned at 70ºC and 80ºC. The unprocessed mash had higher pelleted samples in height and area of CEL ($P\leq0.01$), indicating that pelleting process reduced height and area. How the changes in spectral profiles relate to nutrient values and availability are studied in the next chapter.

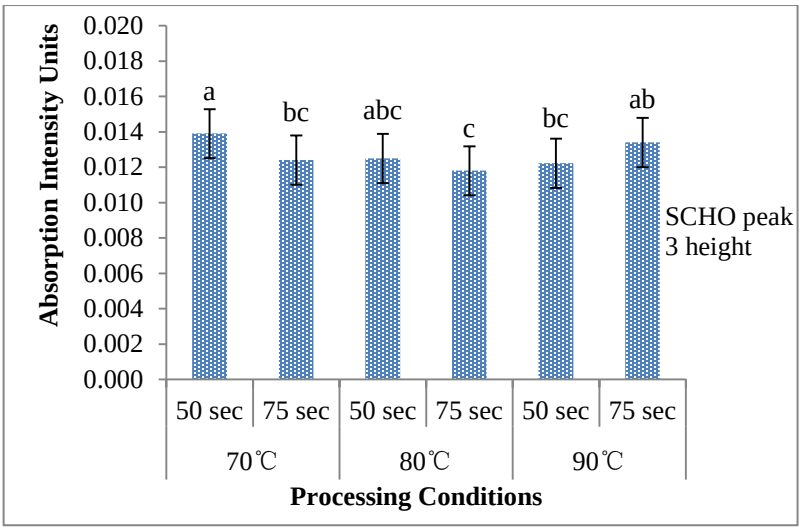


Figure 4.1 Effect of interaction of conditioning temperature and time on structural carbohydrates (SCHO) peak 3 height of canola meal pellets. Comparison among samples on SCHO peak 3 height was set at center range of ca. 1235-1227 cm^{-1} with baseline of ca. 1488-1186 cm^{-1}. Bars with different letters are significantly different ($n=12$; $P<0.05$).

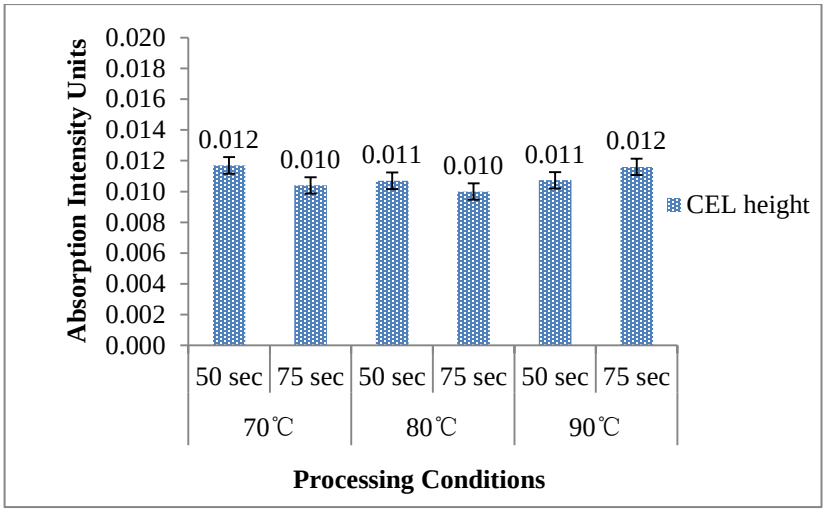


Figure 4.2 Effect of interaction of conditioning temperature and time on cellulosic compounds (CEL) height of canola meal pellets. Comparison among samples on CEL height was set at center range of ca. 1234-1228 cm^{-1} with baseline of ca. 1302-1186 cm^{-1}. Bars with different letters are significantly different (n=12; P<0.05).

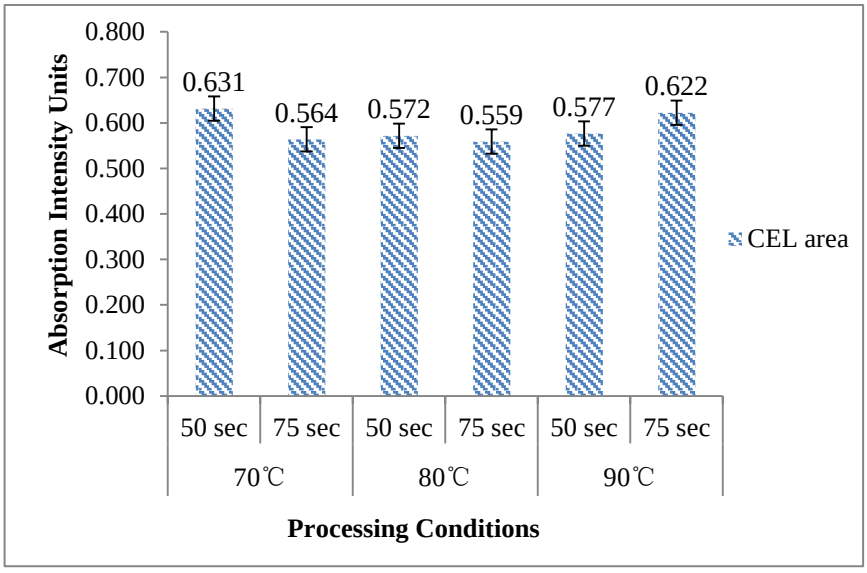


Figure 4.3 Effect of interaction of conditioning temperature and time on cellulosic compounds (CEL) area of canola meal pellets. Comparison among samples on CEL area was set at region and baseline of ca. ca. 1302-1186 cm^{-1}. Bars with different letters are significantly different (n=12; P<0.05)

4.3.1.3. Determining protein molecular spectral characteristics of canola meal at amide I and amide II regions using multivariate analysis

Yu (2005a) indicated that the location of function groups and band patterns determined the accuracy of univariate analysis. For example, it is difficult to distinguish structural CHO and non-structural CHO as their absorption occur at a similar region (ca. 1180-950 cm^{-1}) (Wetzel et al., 1998; Yu, 2005a). Hence, two multivariate analysis methods, Agglomerative Hierarchical Cluster Analysis (CLA) and Principal Component Analysis (PCA), were applied to reveal the molecular structural differences of the unprocessed mash and pellets processed under different conditioning temperatures and time (Yu, 2005a, b, c; Yu, 2008).

Multivariate molecular spectral analysis on the protein spectral region (ca. 1718-1481 cm^{-1}) is shown in the Appendix, Figures 8.2 and 8.3. Figure 8.2 compares protein spectral regions of samples conditioned at different temperatures. Results from CLA indicated that no separate class could be distinguished. No separate class could be distinguished between the samples conditioned at 70ºC and those conditioned at 80ºC (Figure 8.2. (a)). Similarly, as shown in Figure 8.2 (c), the samples conditioned at 70ºC cannot be distinguished from those conditioned at 90ºC due to mixed cluster dendrograms. Figure 8.2 (e) notes that samples conditioned at 80ºC cannot be fully separated from those conditioned at 90ºC. In Figure 8.3 (a), no separate class can be distinguished between the samples conditioned for 50 sec and those conditioned for 75 sec. Therefore, CLA showed similarities of protein structure make-up among pelleted samples processed under different conditions. The samples processed under different conditioning temperatures and time were not completely different in protein structure. This was supported by PCA analysis. In Figures 8.2 (b, d, and f) and 8.3 (b), ellipses were overlapped and cannot be fully separated.

With regard to the difference between the unprocessed mash and the pellets at a protein region of ca. 1718-1481 cm^{-1}, results are shown in Appendix, Figure 8.4. It was observed in that no separate class can be distinguished in CLA (Figure 8.4 (a)), indicating that the unprocessed mash was not completely different from pellets. This was supported by PCA (Figure 8.4 (b)). Though two different ellipses are grouped, two ellipses cannot separate from each other because of the overlap. Comparisons between the unprocessed mash and the pellets produced under different temperatures and time of conditioning, using CLA and PCA, are shown in Figure 8.4 (c-j). The unprocessed mash had similarities to the samples

conditioned at 70ºC in structural make-up within the protein spectral region, because no separate class and ellipse were found in CLA and PCA, respectively (Figure 8.4 (c-d)). Similar results were observed when unprocessed mash was compared with the samples conditioned at 80 and 90ºC, and the samples conditioned for 50 and 75 sec (Figure 8.4 (e-l)). Here, no distinct class can be distinguished in CLA and no grouped ellipse in PCA can be separated. It was therefore summarized above that there were similar relationships between the unprocessed mash and the samples processed under different temperatures and time of conditioning with respect to protein molecular structural make-up.

4.3.1.4. Determining carbohydrate molecular spectral characteristics of pelleted canola meal at TCHO, SCHO, and CEL using multivariate analysis

4.3.1.4.1. Determining carbohydrate molecular spectral characteristics of canola meal at CEL region using multivariate analysis

In this study, cellulosic compounds were detected at ca. 1302-1186 cm^{-1}. The PCA and CLA analyses of structural differences in cellulosic compounds between the unprocessed mash and the samples processed under different temperatures and time of conditioning are presented in the Figure 8.5. Overlapping ellipses and mixed-cluster classes were observed in the comparison between the unprocessed mash and the samples processed under different temperatures and time of conditioning (Figure 8.5 (c-l)), indicating that the unprocessed mash was not fully-distinguished from pellets produced under different conditioning conditions. Paired comparisons among samples processed under different conditions were presented in Figure 8.6. Samples conditioned at 70ºC were not distinguishable from those conditioned at 80 and 90ºC because of mixed-cluster classes in CLA and overlapping ellipses in PCA (Figure 8.6 (c-f)). A similar result was found in comparison between canola meal samples conditioned at 80ºC and those conditioned at 90ºC (Figure 8.6 (g-h). The results of CLA and PCA, with respect to the differences in spectral profiles of cellulosic compounds between the samples conditioned for 50 sec and those conditioned for 75 sec, are shown in Figure 8.6 (i-j). Mixed-cluster classes in CLA and overlapping ellipses in PCA demonstrated that canola meal samples conditioned for 50 sec were not distinguishable from those conditioned for 75 sec in terms of spectral characteristics of cellulosic compounds. In addition, pellets could not be fully-distinguished from the unprocessed mash based on original FTIR spectral of cellulose compounds.

4.3.1.4.2. Determining carbohydrate molecular spectral characteristics of canola meal at SCHO region using multivariate analysis

The results of CLA and PCA of structural CHO (SCHO) based on original spectra at 1488-1186 cm^{-1} are presented in the Appendix, Figure 8.7. The unprocessed mash was not completely distinguishable from samples processed under different conditioning temperatures and time, due to mixed-cluster classes in CLA and heavily-overlapping ellipses in PCA, though more than 95% the variation between the unprocessed mash and pellets can be explained by the first principal components (Figure 8.7 (a-l)). Similar results were observed when comparing the samples processed at different temperatures. Although more than 97% of the variation can be explained by the first principal component, mixed-cluster groups in CLA figures and overlapping ellipses in PCA figures were observed (Figure 8.7 (m-t)). Samples conditioned for 50 sec were not distinguishable in SCHO make-up from those conditioned for 75 sec because of mixed-cluster groups found in CLA and overlapping ellipses found in PCA (Figure 8.7 (u-v)). Hence, there were similarities in SCHO spectral profiles among all samples. In this study, pelleting under different conditioning temperatures and time did not make spectral region of SCHO distinguishable among samples based on original FTIR spectra.

4.3.1.4.3. Determining carbohydrate molecular spectral characteristics of canola meal at TCHO region using multivariate analysis

The total CHO (TCHO) region detected in the current study was at 1193-879 cm^{-1}. Effects of pelleting on spectral characteristics of TCHO based on original spectra, using CLA and PCA, are shown in the Figure 8.8. The unprocessed mash was not completely distinguishable from the samples processed under different temperatures and time of conditioning (Figure 8.8 (a-l)). This was because cluster groups did not form two separate clusters in CLA and ellipses were not grouped separately, though more than 94% of the variation between the unprocessed mash and pellets can be explained by the first principal component in PCA. Similar results were found in comparison when comparing the samples conditioned at different temperatures (Figure 8.8 (m-t)). No separate grouped ellipse in PCA and no clearly separated cluster grouped in CLA was observed, though more than 97% of the variation in TCHO structure among samples processed under different temperatures can be explained by first principal component in PCA. The samples conditioned for 50 sec were not

completely distinguishable in spectral characteristics of TCHO from those conditioned for 75 sec, due to mixed-cluster groups in CLA and overlapping ellipses in PCA (Figure 8.8 (u-v)). Therefore, temperature and time during the conditioning did not make spectral make-up of canola meal at the TCHO region of original spectrum become distinguishable.

There were differences in the results between the univariate statistical analysis and PCA and CLA. This difference was also observed in recent research (Xin and Yu, 2013a). However, it should be noted that band intensities and frequencies of individual components are usually used in univariate analysis. Peak heights and areas only extract partial information about molecular structural characteristics in a specific spectral region and cannot access entire information about the whole spectral region (Xin and Yu, 2013a). The PCA and CLA, which are based on statistical variable reduction, would consider the whole spectral information within the specific region (Xin and Yu, 2013a). In addition, multivariate analysis in my study was based on the original spectrum. Therefore, in our study, although pelleting under different conditions can cause changes in heights, areas, and some spectral ratios of canola meal spectral features, these changes were not sufficient to be picked up by the CLA and PCA multivariate analyses based on original spectrum.

4.4. Conclusion

It can be summarized based on the combined results of univariate and multivariate analyses that pelleting alters inherent structures of functional groups in canola meal. The results of univariate analysis indicated that absorption intensities of functional groups and relative ratios between different absorption bands in canola meal were affected by pelleting. Peak height and area intensities of protein amide II were decreased with increasing conditioning temperature or decreasing conditioning time. Nevertheless, the height and area ratios of amide I to amide II were increased with increasing conditioning temperature but decreased with increasing conditioning time. Spectral profiles of carbohydrate functional groups (TCHO, SCHO and CEL) were affected by pelleting process as well. However, the results of multivariate analysis based on original spectrum indicated that spectral characteristics of functional groups between samples were not fully distinguishable. Pelleting under different temperatures and time of conditioning caused partial changes in spectral characteristics of the functional groups in canola meal, such as peak height and area intensities, but these changes were not sufficient to be picked up by the CLA and PCA

multivariate analyses, and cannot make the entire spectral region of the functional groups fully distinguishable from each other based on original spectrum. The next chapter investigates the relationships between absorption intensities of functional groups (protein, TCHO, SCHO, CEL) and degradation and digestion of protein in canola meal in order to reveal how protein and carbohydrate molecular structures affect nutrient values, rumen degradation and intestinal digestion characteristics, the predicted protein supply of canola meal, and how to determine the most important spectral profiles.

5. Quantify Relationship of Inherent Structure Spectral Profiles to Rumen and Intestinal Digestions and Metabolic Characteristics of the Proteins in Dairy Cattle

5.1. Introduction

Canola meal, which is the byproduct of extracting oil from canola seeds, is widely used as a protein as well as a carbohydrate source for animals. Canola meal is usually traded in pellets or in mash (Newkirk, 2009). Nutrient value, digestion characteristics, and predicted nutrient supply of feed are based not only on its total chemical composition, but also on its inherent structure and component matrices (Yu, 2007). Therefore, understanding the inherent structures of protein and carbohydrates may help explain differences in nutrient values and the availability of different feeds. Digestion characteristics of protein and carbohydrates are affected by their molecular structures (Peng et al., 2014a). On the other hand, carbohydrates and protein need to be considered together when evaluating their digestion characteristics due to interference between them during digestion (Peng et al., 2014a). Recently, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) has been successfully applied in feed research to detect molecular structure and biopolymer conformation using univariate and multivariate analyses. Chapter 4 presented the application of ATR-FTIR to detect differences in protein and carbohydrate structures of canola meal induced by pelleting under different conditioning temperatures and time. It is of interest to detect how protein and carbohydrate molecular structures together affect nutrient values, digestion characteristics and predicted protein supply in canola meal. The objectives of this study were: 1) to quantify the relationship of protein and carbohydrate inherent structure spectral profiles to protein profiles, predicted protein supply, and protein rumen degradation and intestinal digestion characteristics of canola meal; and 2) to determine the most important structural spectral features among protein and carbohydrates in predicting protein profiles, rumen degradation and intestinal digestion characteristics, and predicted protein supply from canola meal to dairy cattle.

5.2. Materials and Methods

5.2.1. Spectral and chemical profiles

The spectral profiles obtained in Chapter 4, related to protein and carbohydrates, were used in the correlation study. The protein spectral profiles include amide I height, amide I area, amide II height, amide II area, ratio of amide I height to amide II height, ratio of amide I area to amide II area, α-helix height, β-sheet height, and ratio of α-helix height and β-sheet height. The carbohydrate functional groups were cellulosic components, structural carbohydrates (SCHO), and total carbohydrates (TCHO). The height and area of cellulosic components (CEL) were used in the correlation study. The SCHO spectral profiles included heights of peak 1, 2, and 3, and total area of SCHO region. Spectral profiles of TCHO included total area region and heights and areas of peak 1, 2, and 3. Protein-related chemical profiles, energy profiles, rumen degradation kinetics and intestinal digestion characteristics, and truly absorbed supply of canola meal to dairy cattle were selected in the correlation study.

5.2.2. Statistical analysis

5.2.2.1. Correlation study between protein molecular structure and nutrient profiles, utilization and availability of canola meal processed under different conditioning temperatures and time

The correlation between spectral profiles of functional groups (CP and CHO) and protein related chemical profiles, energy profiles, rumen degradation kinetics and intestinal digestive characteristics of protein, and truly absorbed protein supply were analyzed using the PROC CORR procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). Rank correlation with the SPEARMAN option and normality test with the UNIVARIATE option were employed in the correlation study.

5.2.2.2. Multiple regression study: protein and carbohydrate molecular structure and nutrient profiles and metabolic characteristics of canola meal processed under conditioning temperatures and time

Multi-regression with PROC REG procedure in SAS 9.3 was used to select the most important parameters. The model used was shown as follows:

$$Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots\ldots\ldots + b_n \times x_n$$

The STEPWISE option with "SLENTRY = 0.05, SLSTAY = 0.05" was used to determine selection criteria. Hence, variables left in the model after selection were significant at $P<0.05$. Then, UNIVARIATE and PLOT options were used to do the residual analysis. VIF option in SAS 9.3 was used to detect the Collinearity, which represented the interrelationship among the protein and carbohydrate structure parameters in our study.

5.3. Results and Discussion

5.3.1. Correlations between protein molecular spectral profiles and nutrient values, digestion characteristics, and predicted nutrient supply of pelleted canola meal

5.3.1.1. Correlations between protein molecular spectral profiles and protein profiles, protein subfractions, and estimated energy profiles in pelleted canola meal

Table 5.1 presents the result of the correlation between protein molecular spectral profiles and protein nutrient profiles, protein subfractions and estimated energy profiles of canola meal. The CP content of canola meal was positively correlated with amide II area ($R = 0.77$, $P<0.05$), while negatively correlated with ratio of amide I area to amide II area ($R = -0.92$, $P<0.01$), indicating that a higher amide II area and a lower area ratio of amide I to amide II were associated with a higher CP content of pelleted canola meal. The SCP content of canola meal was negatively correlated with amide II height ($R = -0.82$, $P<0.05$), but positively correlated with ratio of amide I height to amide II height ($R = 0.89$, $P<0.01$). Hence, a higher amide II height or a lower ratio of amide I height to amide II height was related to a lower SCP content. For protein subfractions, rapidly degradable true protein (PA2) was negatively correlated with amide II height ($R = -0.82$, $P<0.05$), but positively correlated with a ratio of amide I height to amide II height ($R = 0.89$, $P<0.01$). In contrast to PA2, moderately degradable true protein (PB1) was positively correlated with amide II height ($R = 0.85$, $P<0.05$), but negatively correlated with the ratio of amide I height to amide II height ($R = -0.94$, $P<0.01$). Therefore, a higher PA2 and a lower PB1 content was associated with a lower amide II height or a higher ratio of amide I height to amide II height. In addition, PB1 was also positively correlated with amide II area ($R = 0.80$, $P<0.05$) but negatively correlated with the ratio of amide I area to amide II area ($R = -0.92$, $P<0.01$).

Table 5.1 Correlations between protein ATR-FTIR molecular spectral profiles and protein profiles, protein subfractions and estimated energy profiles in pelleting-processed canola meal

Items	Amide I height	Amide II height	Height ratio of amide I to amide II	Amide I area	Amide II area	Area ratio of amide I to amide II	α-helix height	β-sheet height	Ratio of α-helix to β-sheet
				Spearman Correlation R values					
Basic protein profiles									
CP (%DM)	0.30	0.69^{+}	-0.71	0.21	0.77*	-0.92**	0.49	0.25	0.73^{+}
SCP (%CP)	-0.28	-0.82*	0.89**	-0.27	-0.66	0.65	-0.40	-0.20	-0.71^{+}
NDICP (%CP)	0.23	0.37	-0.36	0.23	0.12	0.09	0.20	0.19	0.10
ADICP (%CP)	0.32	0.17	-0.06	0.24	0.12	0.06	0.36	0.34	0.00
Protein subfractions									
PA2 (%CP)	-0.28	-0.82*	0.89**	-0.27	-0.66	0.65	-0.40	-0.20	-0.71*
PB1 (%CP)	0.25	0.85*	-0.94**	0.21	0.80*	-0.92**	0.42	0.16	0.88**
PB2 (%CP)	-0.05	0.11	0.17	0.02	-0.13	0.20	-0.14	-0.09	-0.06
PC (%CP)	0.34	0.13	0.00	0.28	0.06	0.17	0.35	0.37	-0.09
TP (%CP)	-0.34	-0.12	-0.01	-0.23	-0.06	-0.17	-0.35	-0.37	0.10
PA2_TP (%CP)	-0.27	-0.81*	0.90**	-0.25	-0.66	0.66	-0.38	-0.17	-0.72^{+}
PB1_TP (%CP)	0.33	0.88**	-0.94**	0.28	0.83*	-0.89**	0.51	0.25	0.87*
PB2_TP (%CP)	-0.03	0.11	-0.17	0.04	-0.13	0.27	-0.12	-0.07	-0.07
Estimated energy profiles									
tdCP (%DM)	0.26	0.68	-0.72	0.18	0.77*	-0.94*	0.44	0.20	0.75
TDN_{1x}, %DM	0.56	0.03	0.27	0.55	0.15	0.25	0.53	0.64	-0.32
TDN_{3x}, %DM	0.55	0.02	0.27	0.54	0.13	0.27	0.51	0.63	-0.34
DE_{1x}, Mcal/kg,	0.52	0.12	0.12	0.50	0.28	0.03	0.54	0.59	-0.16
DE_{p3x}, Mcal/kg	0.34	0.53	-0.49	0.32	0.64	-0.62	0.45	0.35	0.28
ME_{p3x}, Mcal/kg	0.45	0.44	-0.33	0.40	0.57	-0.46	0.58	0.47	0.33
NE_{Lp3x}, Mcal/kg	0.44	0.61	-0.55	0.33	0.58	-0.52	0.59	0.40	0.59
ME, Mcal/kg,	-0.53	-0.46	0.31	-0.61	-0.41	0.01	-0.47	-0.60	0.27
NE_m, Mcal/kg,	0.49	0.50	-0.37	0.38	0.56	-0.47	0.60	0.48	0.37
NE_g, Mcal/kg,	0.38	0.12	0.04	0.31	0.22	-0.04	0.48	0.41	0.24

Notes: n=14. R: correlation coefficient calculated using Spearman method; CP: crude protein; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; SCP: soluble crude protein; PA2: rapidly degradable true protein; PB1: moderately degradable true protein. PB2: slowly degradable true protein; PC: undegradable protein; TP: true protein; PA2_TP: PA2 presents in %TP basis;

Table 5.1 Cont'd.

PB1: PB1 presents in %TP basis; PB2_TP: PB2 presents in %TP basis; tdCP: total digestible crude protein; TDN_{1x}: total digestible nutrients; TDN_{3x}: total digestible nutrients at 3x maintenance; DE_{1x}: digestible energy; DE_{p3x}: digestible energy at a production level (3x maintenance); ME_{p3x}: metabolizable energy at a production level (3x maintenance); NE_{Lp3x}: net energy at a production level (3x maintenance); ME: metabolizable energy; NE_m: net energy for maintenance; NE_g: net energy for gain. "+": $P<0.10$; "*": $P<0.05$ "**": $P<0.01$.

Therefore, a lower PB1 content also related to a lower amide II area and a lower ratio of amide I area to amide II area. In estimated energy profiles, only total digestible protein (tdCP) was observed as positively correlated with amide II area ($R = 0.77$, $P<0.05$), but negatively correlated with the ratio of amide I area to amide II area ($R = -0.94$, $P<0.05$). It is concluded that the amide II peak has an important relationship with nutrient profiles of pelleted canola meal. Yan et al. (2014) found that protein amide peaks and protein secondary structures (α-helix and β-sheet) have important relationships with protein profiles and subfractions of microwaved barley (2450 MHz; 0, 3, and 5 mins).

5.3.1.2. Correlations between protein spectral profiles and rumen degradation and intestinal digestion characteristics of pelleted canola meal

Correlations between protein spectral profiles and rumen degradation and intestinal digestion characteristics of pelleted canola meal, are shown in Table 5.2. The soluble fraction (S) of CP positively correlated with amide II height ($R = 0.76$, $P<0.05$) and amide II area ($R = 0.77$, $P<0.05$), but negatively correlated with the ratio of amide I height to amide II height ($R = -0.80$, $P<0.05$) and ratio of amide I area to amide II area ($R = -0.88$, $P<0.01$). This indicated that the amide II peak had important relationship with S of CP in pelleted canola meal. A strong correlation ($R = 0.78$, $P<0.05$) was observed between degradable fraction (D) of CP and the ratio of amide I area to amide II area, indicating a higher D of CP was associated with a higher ratio of amide I area to amide II area. Therefore, the results indicated that changes in rumen degradation of CP may be contributed by the differences in the protein molecular structure. Several publications supported that protein amide peaks were linked to rumen degradation characteristics of CP (Liu et al., 2012; Xin et al., 2013; Yan et al., 2014). It was exhibited that total digestible protein (TDP) of pelleted canola meal was positively correlated with area and height of amide II ($R = 0.80$, $P<0.05$, $R = 0.80$, $P<0.05$, respectively). Hence, a higher amide II height and area was associated with a higher TDP. However, insignificant correlations ($P>0.05$) between protein amide peaks and intestinal digestion characteristics suggested that spectral profiles protein amide peaks were not associated with intestinal digestion of protein in our study. No rumen degradation and intestinal digestion parameter of pelleted canola meal was significantly correlated with the protein's secondary structure (α-helix, β-sheet, and ratio of α-helix to β-sheet) in this study.

Table 5.2 Correlations between protein ATR-FTIR spectral profiles and rumen degradation and intestinal digestion characteristics of pelleting-processed canola meal

Items	Amide I height	Amide II height	Height ratio of amide I to amide II	Amide I area	Amide II area	Area ratio of amide I to amide II	α-helix height	β-sheet height	Ratio of α-helix to β-sheet
				Spearman Correlation R values					
Rumen degradation kinetics of protein									
K_d (%/h)	-0.33	-0.07	-0.10	-0.31	0.04	-0.32	-0.26	0.30	-0.07
T_0 (h)	-0.47	-0.08	-0.18	-0.38	-0.10	-0.13	-0.44	-0.45	-0.15
S (%CP)	0.26	0.76*	-0.82*	0.22	0.77*	-0.88**	0.40	0.21	0.56
D (%CP)	-0.12	-0.51	0.58	-0.09	-0.61	0.78*	-0.24	-0.10	-0.41
U (%CP)	-0.29	-0.39	0.36	-0.27	-0.18	-0.03	-0.30	-0.25	-0.22
%BCP (%CP)	0.05	-0.43	0.58	0.06	-0.48	0.70^{+}	-0.09	0.06	-0.33
RUP^{NRC} (g/kg, DM)	0.09	-0.38	0.54	0.10	-0.44	0.07^{+}	-0.05	0.10	-0.32
BCP^{DVE} (g/kg, DM	0.09	-0.38	0.51	0.10	-0.44	0.68^{+}	-0.05	0.10	0.32
%EDCP (%CP)	-0.05	0.43	-0.58	-0.06	0.48	-0.70+	0.09	-0.06	0.33
EDCP (g/kg, DM)	0.03	0.51	-0.64	0.02	0.51	0.02^{+}	0.54	0.16	-0.72+
Intestinal digestion of protein									
%dIDP (%RUP)	0.40	-0.13	0.39	0.45	-0.14	0.57	0.21	0.41	-0.38
IDP (%RUP)	0.18	-0.34	0.52	0.20	-0.38	0.63^{+}	0.02	0.19	-0.37
IDP (g/kg DM)	0.20	-0.30	0.49	0.22	-0.36	0.68^{+}	0.03	0.21	-0.36
TDP (%CP)	0.46	0.54	-0.42	0.53	0.60	-0.37	0.42	0.46	0.00
TDP (g/kg,DM)	0.62	0.80*	-0.68	0.66	0.80*	-0.54	0.63	0.60	0.22

Notes: n=14. R: Correlation coefficient calculated using Spearman method; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T0: lag time in the rumen; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in NRC Dairy 2001 model; EDCP: effectively degraded crude protein; IDP: intestinal digestible protein; dIDP: intestinal digestibility of rumen undegraded protein; RUP: rumen undegraded protein; TDP: total digestible protein. "+": P<0.1; "*": P<0.05 "**": P<0.010.

Samadi et al. (2013) observed that the ratio of α-helix to β-sheet was significantly correlated with rumen degradation and intestinal digestion characteristics of protein in autoclaved (120ºC for 1 h) and roasted (120ºC for 1 h) canola seeds. Theodoridou and Yu (2013a) also observed protein secondary structure had important relationships with protein rumen degradation kinetics (Kd, S, D, effectively degraded CP and rumen undegradable CP) and intestinal degradation characteristics in canola meal and canola presscake. Yu (2005c) reported that lower nutrient availability was induced by a higher β-sheet content. The reason for the difference between our study and previous studies is likely due to the application of different processing methods and conditions.

5.3.1.3. Correlations between protein spectral profiles and predicted nutrient supply, based on the NRC 2001 models, in pelleted canola meal

Correlations between protein spectral profiles and truly absorbed protein supply to dairy cattle using the NRC 2001 models in pelleted canola meal are shown in Table 5.3. Microbial protein synthesized in the rumen based on discounted TDN (MCP_{TDN}) was strongly negatively correlated with the ratio of α-helix to β-sheet ($R = -0.81$, $P<0.05$). This indicated a higher MCP_{TDN} was associated with a higher ratio of α-helix to β-sheet in our study. Changes in protein secondary structure may partially influence microbial protein production. A similar result was observed in the correlation between microbial protein based on NRC (2001) (MCP^{NRC}) and the ratio of α-helix to β-sheet ($R=-0.81$, $P<0.05$). Correlation between AMCP and spectral profiles of different protein functional groups were not significant ($P>0.05$). Rumen endogenous protein (ECP) was positively correlated with the ratios of amide I height to amide II height and amide I area to amide II area ($R = 0.81$, $P<0.05$, $R = 0.94$, $P<0.01$, respectively). A higher ECP content was associated with the higher ratios. A similar result was detected in the study of the correlation between $AECP^{NRC}$ and the ratio of amide I height to amide II height ($R = 0.82$, $P<0.05$), and between $AECP^{NRC}$ and the ratio of amide I area to amide II area ($R = 0.93$, $P<0.01$). A higher $AECP^{NRC}$ content was associated with higher height and area ratios of amide I to amide II.

Table 5.3 Correlations between protein ATR-FTIR spectral profiles and predicted nutrients supply based on NRC 2001 models in pelleting-processed canola meal

Items (g/kg, DM)	Amide I height	Amide II height	Height ratio of amide I to amide II	Amide I area	Amide II area	Area ratio of amide I to amide II	α-helix height	β-sheet height	Ratio of α-helix to β-sheet
				Spearman Correlation R values					
Estimation of nutrient supply using the NRC 2001 model									
MCP_{TDN}	0.26	-0.17	0.36	0.35	-0.24	0.65	0.05	0.33	-0.81*
MCP^{NRC}	0.26	-0.17	0.36	0.35	-0.24	0.65	0.05	0.33	-0.81*
$AMCP^{NRC}$	0.54	-0.08	0.37	0.56	-0.05	0.56	0.42	0.62	-0.55
RUP^{NRC}	0.10	-0.38	0.53	0.10	-0.44	0.68^{+}	-0.04	0.10	-0.32
$ARUP^{NRC}$	0.20	-0.30	0.49	0.22	-0.36	0.68^{+}	0.03	0.21	-0.36
ECP	-0.14	-0.70^{+}	0.81*	-0.07	-0.72^{+}	0.94**	-0.33	-0.08	-0.73^{+}
$AECP^{NRC}$	-0.14	-0.71^{+}	0.82*	-0.08	-0.73^{+}	0.93**	-0.34	-0.09	-0.72^{+}
MP^{NRC}	0.20	-0.30	0.49	0.22	-0.36	0.68^{+}	0.03	0.21	-0.36
OEB^{NRC}	0.01	0.49	-0.62	0.00	0.54	-0.74^{+}	0.15	-0.01	0.38
FMV	0.20	-0.31	0.50	0.20	-0.35	0.70^{+}	0.04	0.20	-0.35

Notes: n=14. R: Correlation coefficient calculated using Spearman method; FOM: fermented organic matter in the rumen; TPSI: total protein supplied to the small intestine; ENDP: endogenous protein in the small intestine; N_MCP: microbial protein synthesized based on available nitrogen; $AMCP^{DVE}$: truly absorbed microbial protein in the small intestine; BCP: rumen bypass protein; $ABCP^{DVE}$: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance in DVE/OEB model; MCP_{TDN}: microbial protein synthesized in the rumen based on discounted TDN; MCP^{NRC}: microbial protein; $AMCP^{NRC}$: truly absorbed microbial protein in the small intestine; RUP^{NRC}: rumen undegradable feed crude protein; $ARUP^{NRC}$: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; $AECP^{NRC}$: truly absorbed rumen endogenous protein in the small intestine; MP^{NRC}: metabolizable protein; FMV: Feed Milk Value; OEB^{NRC}: degraded protein balance in the NRC 2001 model. "+": $P<0.10$; "*": $P<0.05$ "**": $P<0.01$.

However, MP^{NRC} and OEB^{NRC} were not significantly correlated with protein spectral profiles ($P>0.05$). Liu et al. (2012) found that MP^{NRC} and OEB^{NRC} in cereal grains were correlated with spectral profiles of amide peaks and protein secondary structure. Theodoridou and Yu (2013a) observed that protein secondary structure was significantly correlated with MP^{NRC} and OEB^{NRC} in canola meal and canola presscake. The reasons for the difference between our study and published data are likely due to feed types and processing conditions. Peng et al. (2014a) observed that carbohydrate spectral profiles were correlated with rumen degradation kinetics of protein in different common prairie feeds. Therefore, it would be interesting to study the correlation between spectral profiles of carbohydrates and nutrient profiles, rumen degradation and intestinal digestion of protein, and protein supply of pelleted canola meal (Table. 8.1-8.3).

5.3.2. Multi-regression to detect the most important variables among protein and carbohydrate spectral profiles in predicting nutrient profiles, rumen degradation and intestinal digestion characteristics, and truly absorbed nutrient supply of canola meal

Tables 5.4-5.6 show the results of multi-regression with spectral variables selection. The model used in multi-regression was: Y = amide I height (AIH) + amide II area (AIIA) + amide I area (AIA) + amide II height (AIIH) + ratio of amide I to amide II area (ratio1) + ratio of amide I to amide II height (ratio3) + α-helix (HH) + β-sheet (SH) + ratio of α-helix to β-sheet (ratio2) + cellulosic compounds area (CA) + cellulosic compounds height (CH) + SCHO peak 1 height (SCP1) + SCHO peak 2 height (SCP2) + SCHO peak 3 height (SCP3) + SCHO area (SCA) + TCHO peak 1 height (TCP1H) + TCHO peak 2 height (TCP2H) + TCHO peak 3 height (TCP3H) + TCHO peak 1 area (TCP1A) + TCHO peak 2 area (TCP2A) + TCHO 3 area (TCP3A) + TCHO area (TCA). Peng et al. (2014a) indicated that protein and carbohydrate should not be considered separately when assessing protein digestion. This is because carbohydrate and protein would interfere with each other during the process of digestion. Therefore, this analysis was designed to select the most important protein and carbohydrate structural variables for determining protein profiles, rumen degradation and intestinal digestion characteristics of protein, and protein supply of pelleted canola meal.

The results of multi-regression analysis for protein nutritional profiles are shown in Table 5.4. The ratio of amide I to amide II area was a better predictor for CP, making up 84% of the total variance.

Table 5.4 Multi-regression analysis using ATR-FTIR with tested regression model to detect the most important variables among protein and carbohydrate spectral profiles in predicting nutrients profiles of canola meal

Variables (Y)	Variables in the model with P<0.05	Equation prediction. $Y = a + b_1 \times x_1 + b_2 \times x_2 + \ldots\ldots\ldots$	R^2 values	RSD	P values
Protein profiles					
CP (%DM)	Ratio1stayed in the model	CP = 44.66 -1.96 × ratio1	0.84	0.12	<0.01
SCP (%CP)	Ratio3 stayed in the model	SCP = 8.61 + 8.20 × ratio3	0.80	0.43	<0.01
NDICP (%CP)	No variable met the 0.05 significance level for entry into the model.				
ADICP (%CP)	No variable met the 0.05 significance level for entry into the model.				
Protein subfractions					
PA2 (%CP)	Ratio3 stayed in the model	PA2 = 8.61 + 8.20 × ratio3	0.80	0.43	<0.01
PB1 (%CP)	SCP2, SCA and ratio3 stayed in the model	PB1 = 71.80 - 547.76 × SCP2+1.80 × SCA - 5.32× ratio3	0.9996	0.02	<0.0001
PB2 (%CP)	No variable met the 0.05 significance level for entry into the model.				
PC (%CP)	No variable met the 0.05 significance level for entry into the model.				
TP (%CP)	No variable met the 0.05 significance level for entry into the model.				
PA2_TP (%TP)	ratio3 stayed in the model	PA2_TP = 8.96+8.62 × ratio3	0.81	0.45	<0.01
PB1_TP (%TP)	ratio3 stayed in the model	PB1_TP = 77.51-7.92 × ratio3	0.90	0.28	<0.01
PB2_TP (%TP)	No variable met the 0.05 significance level for entry into the model.				
Estimated energy profiles					
tdCP (%DM)	ratio1 stayed in the model	tdCP = 43.98 - 1.97 × ratio1	0.89	0.10	<0.01
TDN_{1x} (%DM)	No variable met the 0.05 significance level for entry into the model.				
TDN_{3x} (%DM)	No variable met the 0.05 significance level for entry into the model.				
DE_{1x}, Mcal/kg, dairy	No variable met the 0.05 significance level for entry into the model.				

Table 5.4 Cont'd.

DE_{p3x}, Mcal/kg, dairy	TCP2H stayed in the model	DE_{p3x} = 3.19 - 0.40421 × TCP2H	0.58	0.003	<0.05
ME_{p3x}, Mcal/kg, dairy	No variable met the 0.05 significance level for entry into the model.				
NE_{lp3x}, Mcal/kg, dairy	No variable met the 0.05 significance level for entry into the model.				
ME, Mcal/kg, beef	CA and TCP2H stayed in the model	ME = 2.70 - 0.22 × CA + 3.31 × TCP2H	0.98	0.01	<0.001

Notes: n=14. RSD: Residual standard deviation; CP: crude protein; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; SCP: soluble crude protein; PA2: rapidly degradable true protein; PB1: moderately degradable true protein. PB2: slowly degradable true protein; PC: undegradable protein; TP: true protein; PA2_TP: PA2 presents in %TP basis; PB1: PB1 presents in %TP basis; PB2_TP: PB2 presents in %TP basis; tdCP: total digestible crude protein; TDN_{1x}: total digestible nutrients; TDN_{3x}: total digestible nutrients at 3x maintenance; DE1x: digestible energy; DE_{p3x}: digestible energy at a production level (3x maintenance); ME_{p3x}: metabolizable energy at a production level (3x maintenance); NEL_{p3x}: Net energy at a production level (3x maintenance); ME: metabolizable energy; NE_m: net energy for maintenance; NE_g: net energy for gain. Ratio1: ratio of amide I to amide II area; ratio3: ratio of amide I to amide II height; SCP2: structural carbohydrates peak 2 height; SCA: total structural carbohydrate area; TCP2H: total carbohydrates peak 2 height; CA: cellulosic compounds area. Baseline for protein spectral peak: ca. 1481-1718 cm^{-1}; protein amide I region: ca. 1718-1567 cm^{-1}; protein amide II region: ca. 1567-1481 cm^{-1}; center range of amide I peak: ca. 1644-1625 cm^{-1}; center range of amide II peak: ca. 1538-1535 cm^{-1}; center range for α-helix: ca. 1660-1650 cm^{-1}; center range for β-sheet: ca. 1627-1618 cm^{-1}. Total carbohydrate baseline and region: ca.1193-879 cm^{-1}; total carbohydrate peak 1 region: ca. 1192-1126 cm^{-1}; total carbohydrate peak 1 center range: ca.1159-1150 cm^{-1}; total carbohydrate peak 2 region: ca. 1126-990 cm^{-1}; total carbohydrate peak 2 center range: ca. 1170-1103 cm^{-1}; total carbohydrate peak 3 region: ca. 990-881 cm^{-1}. Baseline for structural carbohydrates: ca. 1488-1186 cm^{-1}; structural carbohydrate peak1 height center range: ca. 1417-1409 cm^{-1}; structural carbohydrates peak 2 height center range: ca. 1393-1367 cm^{-1}; structural carbohydrate peak 3 height center range: ca. 1235-1227 cm^{-1}. Baseline and region for cellulosic compounds: 1302-1186 cm^{-1}; cellulosic height center range: ca. 1234-1228 cm^{-1}.

This was in accordance with Liu et al. (2012) on different cereal grains (wheat, corn and triticale) and their dried distillers grains with solubles (DDGS). The ratio of amide I to amide II area was the predictor left in the model for CP and made up 79% of total variance in their study. The ratio of amide I to amide II height was the selected predictor for SCP, making up 80% of the total variance. In protein subfractions, the ratio of amide I to amide II height was the better predictor for PA2, PA2_TP and PB1_TP, and made up 80%, 81% and 90% of the total variance, respectively. The SCHO peak 2 height, SCHO area, and ratio of amide I to amide II height together made up 99.96% of the variance in the model for PB1. Therefore, PB1 can be estimated based on spectral profiles of protein and carbohydrate. In energy estimation, ratio of amide I to amide II area was the only parameter left in the model for tdCP and accounted for 89% of the total variance. This was similar to Yu and Nuez-Ortín (2010) and Liu et al. (2012). Yu and Nuez-Ortín (2010) studied on different DDGS and found that ratio of amide I to made II area was the predictor for tdCP and accounted for 91% of total variance. Liu et al. (2012) found ratio of amide I to made II area was the only one left in the model for tdCP and accounted for 79% of total variance. The TCHO peak 2 height was the only predictor left in the model for DE_{p3x}, but only accounted for 58% of the total variance. The cellulosic compounds area and TCHO peak 2 height were left in the model and made up 98% of the total variance in predicting ME, indicating that ME can be estimated using spectral profiles of carbohydrate.

Results of multi-regression analysis for rumen degradation and intestinal digestion of protein are shown in Table 5.5. Ratio of amide I to amide II area and ratio of α-helix to β-sheet were selected as predictors for S of protein, and accounted for 96% of the total variance. The TCHO peak 2 height and TCHO peak 1 area were better predictors for pBCP, RUP, BCP, pEDCP, EDCP, pIDP, and IDP, accounting for 93%, 92%, 92%, 93%, 94%, 97%, and 96% of the total variance, respectively. The higher the total variance predictors take, the higher variance in the model can be explained by predictors. The TCHO 3 area was the only parameter left in the model and accounted for 73% of the total variance in the prediction of pdIDP. The amide II area was the better predictor for TDP, accounting for 65% of the total variance. Compared with studies that used either protein or carbohydrate spectral profiles assessing protein digestion, our study considered the interference between protein and carbohydrates.

Table 5.5 Multi-regression analysis using ATR-FTIR with tested regression model to detect the most important variables among protein and carbohydrate spectral profiles in correlating rumen degradation and intestinal digestion characteristics of canola meal

Variables (Y)	Variables in the model with P<0.05	Equation prediction. $Y = a + b_1 \times x_1 + b_2 \times x_2$	R^2 values	RSD	P values
Rumen degradation kinetics of protein					
K_d (%/h)	No variable met the 0.05 significance level for entry into the model.				
T0 (h)	No variable met the 0.05 significance level for entry into the model.				
S(%CP)	ratio1 and ratio2 stayed in the model	S = 235.05 - 32.48 × ratio1 - 145.93 × ratio2	0.96	0.61	<0.01
D(%CP)	No variable met the 0.05 significance level for entry into the model.				
U(%CP)	No variable met the 0.05 significance level for entry into the model.				
pBCP	TCP2H and TCP1A stayed in the model	pBCP = -4.88 +145.28 × TCP2H + 37.08 × TCP1A	0.93	0.83	<0.01
RUP	TCP2H and TCP1A stayed in the model	RUP = - 16.42 + 532.19 × TCP2H + 147.57 × TCP1A	0.92	3.40	<0.01
BCP	TCP2H and TCP1A stayed in the model	BCP = - 18.21 + 590.75 × TCP2H + 163.79 × TCP1A	0.92	3.78	<0.01
pEDCP	TCP2H and TCP1A stayed in the model	pEDCP = 104.82 - 145.31 × TCP2H - 37.02 × TCP1A	0.93	0.83	<0.01
EDCP	TCP2H and TCP1A stayed in the model	EDCP = 411.24 - 654.86 × TCP2H - 136.09 × TCP1A	0.94	2.90	<0.01
Intestinal digestion of protein					
pdIDP (%RUP)	TCP3A stayed the model	pdIDP = 35.35 + 2.74 × TCP3A	0.73	1.12	<0.05
pIDP (%RUP)	TCP2H and TCP1A stayed in the model	pIDP = - 18.30 + 101.20 × TCP2H + 37.30 × TCP1A	0.97	0.50	<0.01
IDP (g/kg,DM)	TCP2H and TCP1A stayed in the model	IDP = - 70.35 + 376.22 × TCP2H + 147.74 × TCP1A	0.96	2.19	<0.01
pTDP (%CP)	No variable met the 0.05 significance level for entry into the model.				
TDP (g/kg,DM)	AIIA stayed in the model	TDP = 301.67 + 17.82 × AIIA	0.65	2.06	<0.05

Notes: n=14. RSD: Residual standard deviation; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T0: lag time in the rumen; S: soluble fraction in the in situ incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded crude protein; IDP: intestinal digestible protein; dIDP: intestinal digestibility of rumen undegraded protein; RUP: rumen undegraded protein; TDP: total digestible protein; ratio1: ratio of amide I to amide II area; ratio2: ratio of α-helix to β-sheet height; TCP2H: total carbohydrates peak 2 height; TCP1A: total carbohydrates peak 1 area; TCP3A: total carbohydrates peak 3 area; AIIA: amide II area. Baseline for protein spectral peak: ca. 1481-1718 cm^{-1}; protein amide I region: ca. 1718-1567 cm^{-1}; protein amide II region: ca. 1567-1481 cm^{-1}; center range of amide I peak: ca. 1644-1625 cm^{-1}; center range of amide II peak: ca. 1538-1535 cm^{-1}; center range for α-helix: ca. 1660-1650 cm^{-1}; center range for β-sheet: ca. 1627-1618 cm^{-1}. Total carbohydrate baseline and region: ca.1193-879 cm^{-1}; total carbohydrate peak 1 region: ca. 1192-1126 cm^{-1};

Table 5.5 Cont'd.

total carbohydrate peak 1 center range: ca.1159-1150 cm^{-1}; total carbohydrate peak 2 region: ca. 1126-990 cm^{-1}; total carbohydrate peak 2 center range: ca. 1170-1103 cm^{-1}; total carbohydrate peak 3 region: ca. 990-881 cm^{-1}. Baseline for structural carbohydrates: ca. 1488-1186 cm^{-1}; structural carbohydrate peak1 height center range: ca. 1417-1409 cm^{-1}; structural carbohydrates peak 2 height center range: ca. 1393-1367 cm^{-1}; structural carbohydrate peak 3 height center range: ca. 1235-1227 cm^{-1}. Baseline and region for cellulosic compounds: 1302-1186 cm^{-1}; cellulosic height center range: ca. 1234-1228 cm^{-1}.

Table 5.6 Multi-regression analysis using ATR-FTIR with tested regression model to detect the most important variables among protein and carbohydrate spectral profiles in correlating predicted nutrients supply

Variables (Y)	Variables in the model with P<0.05	Equation prediction. $Y = a + b_1 \times x_1 + b_2 \times x_2$	R^2 values	RSD	P values
Predicted nutrients using the NRC 2001 model (g/kg DM)					
MCP_{TDN}	SCP2 stayed in the model	MCP_{TDN} = 80.50 + 126.11 × SCP2	0.90	0.05	<0.01
MCP^{NRC}	SCP2 stayed in the model	MCP^{NRC} = 80.50 + 126.11 × SCP2	0.90	0.05	<0.01
$AMCP^{NRC}$	CA stayed in the model	$AMCP^{NRC}$ = 52.55 + 1.95 × CA	0.64	0.06	<0.05
RUP^{NRC}	TCP2H and TCP1A stayed in the model	RUP^{NRC} = -20.13 + 543.18 × TCP2H + 150.44 × TCP1A	0.92	3.46	<0.01
$ARUP^{NRC}$	TCP2H and TCP1A stayed in the model	$ARUP^{NRC}$ = -70.35 + 376.22 × TCP2H + 147.74 × TCP1A	0.96	2.19	<0.01
ECP	ratio1 stayed in the model	ECP = 4.48 + 2.45 × ratio1	0.88	0.13	<0.01
$AECP^{NRC}$	ratio1 stayed in the model	$AECP^{NRC}$ = 1.82 + 0.97 × ratio1	0.87	0.05	<0.01
MP^{NRC}	TCP1A and TCP2H stayed in the model	MP^{NRC} =-17.03+376.61×TCP2H+151.14×TCP1A	0.96	2.18	<0.01
OEB^{NRC}	TCP1A and TCP2H stayed in the model	OEB^{NRC} = 330.12 - 153.16 × TCP1A - 637.13 × TCP2H	0.93	3.49	<0.01
Feed Milk Value					
FMV	TCP2H and TCP1A stayed in the model	FMV = -0.35+7.56*TCP2H+3.08*TCP1A	0.96	0.05	<0.01

Notes: n=14. RSD: Residual standard deviation; FMV: Feed Milk Value; MCP_{TDN}: microbial protein synthesized in the rumen based on discounted TDN; MCP^{NRC}: microbial protein; $AMCP^{NRC}$: truly absorbed microbial protein in the small intestine; RUP^{NRC}: rumen undegradable feed crude protein; $ARUP^{NRC}$: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; $AECP^{NRC}$: truly absorbed rumen endogenous protein in the small intestine; MP^{NRC}: metabolizable protein; OEB^{NRC}: degraded protein balance in the NRC 2001 model; SCP2: structural carbohydrates peak 2 height; CA: cellulosic compounds area; TCP2H: total carbohydrates peak 2 height; TCP1A: total carbohydrates peak 1 area; SCP1: structural carbohydrates peak 1 area. Baseline for protein spectral peak: ca. 1481-1718 cm^{-1}; protein amide I region: ca. 1718-1567 cm^{-1}; protein amide II region: ca. 1567-1481 cm^{-1}; center range of amide I peak: ca. 1644-1625 cm^{-1}; center range of amide II peak: ca. 1538-1535 cm^{-1}; center range for α-helix: ca. 1660-1650 cm^{-1}; center range for β-sheet: ca. 1627-1618 cm^{-1}. Total carbohydrate baseline and region: ca.1193-879 cm^{-1}; total carbohydrate peak 1 region: ca. 1192-1126 cm^{-1}; total carbohydrate peak 1 center range: ca.1159-1150 cm^{-1}; total carbohydrate peak 2 region: ca. 1126-990 cm^{-1}; total carbohydrate peak 2 center range: ca. 1170-1103 cm^{-1}; total carbohydrate peak 3 region: ca. 990-881 cm^{-1}. Baseline for structural carbohydrates: ca. 1488-1186 cm^{-1}; structural carbohydrate peak1 height center range: ca. 1417-1409 cm^{-1}; structural carbohydrates peak 2 height center range: ca. 1393-1367 cm^{-1}; structural carbohydrate peak 3 height center range: ca. 1235-1227 cm^{-1}. Baseline and region for cellulosic compounds: 1302-1186 cm^{-1}; cellulosic height center range: ca. 1234-1228 cm^{-1}.

Results of multi-regression analysis for predicted protein supply based on the NRC 2001 model are shown in Table 5.6. The SCHO peak 2 height was the only variable left in models for MCP_{TDN} and MCP^{NRC}, accounting in each 90% of the total variance. The cellulosic compounds area was the better predictor for $AMCP^{NRC}$ and accounted for 64% of the total variance. TCHO peak 2 height and TCHO peak 1 area were the predictors for RUPNRC (with 92 % of the variance being accounted for), $ARUP^{NRC}$ (with 96 % of the variance being accounted for), MP^{NRC} (with 96% of the variance being accounted for) and OEBNRC (with 93 % of the variance being accounted for). Ratio of amide I to amide II area was the only one parameter left in the models for ECP and $AECP^{NRC}$, and accounted for 88% and 87% of the total variance, respectively. In our study, none of protein spectral profiles was selected in predicting MP^{NRC} and OEB^{NRC}, this was different from Liu et al. (2012) and Peng et al. (2014). In their study, they used protein spectral profiles to predict protein supply of feeds.

5.4. Conclusion

In summary, this study observed that protein molecular spectral profiles correlate with protein nutritive profiles, protein subfractions, rumen degradation kinetics and intestinal digestion characteristics of protein, and protein supply of pelleted canola meal. The result of multi-regression analyses demonstrates that based on protein and carbohydrate spectral profiles, predictions of protein profiles, rumen degradation and intestinal digestion characteristics of protein, and predicted protein supply are possible. For protein profiles of canola meal, CP content can be estimated as: 44.66 - 1.96 × ratio of amide I to made II area (R^2 = 0.84). For predicted protein supply based on NRC-2001 model, metabolizable protein (MP^{NRC}) can be estimated as: -17.03 + 376.61 × TCHO peak 2 height + 151.14 × TCHO peak 1 area (R^2 = 0.96). This approach provides a new way at its preliminary stage to estimate protein availability of feeds based on the molecular structure of protein and carbohydrates. It also helps researchers understand the interactional effect of protein and carbohydrates on protein utilization and availability. Therefore, developing a new method for estimating protein values rapidly without complex analytical procedures will be beneficial to research. It should be kept in mind that determination of the applicability of this predicted model requires extensive *in vivo* studies (Yu and Nuez-Ortín, 2010; Theodoridou and Yu, 2013b). In the future, experiments with more different levels of conditioning temperature and time should be conducted to determine the applicability of predicted models in field.

6. General Discussion, Overall Conclusion, and Future Research

A high protein content coupled with excellent protein quality makes canola meal a premium protein source for animals (Newkirk, 2009). Up to 60% of the metabolizable protein required for milk production in dairy cows can be made up of microbial protein. Canola meal has a high rumen degradability and can supply sufficient rumen degradable protein (RDP) to provide ammonia, peptides, and amino acids for synthesis of microbial protein and growth of microbes in the rumen (Newkirk, 2009).

Canola meal not only can support high milk production, but also results in high milk protein quality (Newkirk, 2009). However, the relatively low energy level and high fiber content may limit the use of canola meal in animal feed.

This study investigated effects of conditioning temperature and time on nutritional profiles, rumen degradation and intestinal digestion characteristics, and predicted protein supply of canola meal. Application of ATR-FTIR in investigating changes in protein inherent structure induced by different conditioning temperatures and time during pelleting was also conducted. In Chapter 3, the pellet durability test revealed that longer conditioning time resulted in a higher pellet durability index (PDI), while altering the conditioning temperature did not have significant effects on PDI. In terms of chemical profiles, the samples processed for a longer conditioning time had higher neutral detergent insoluble crude protein (NDICP), but low soluble CP (SCP) content. For energy values, there was no difference among all samples, suggesting that of energy values of canola meal were not modified by pelleting with different conditioning temperatures and time. With respect to protein subfractions of canola meal, rapidly degradable true protein (PA2) content was decreased with increasing conditioning time, while the effect of conditioning temperature was not significant. In addition, our study observed found slowly degradable true protein (PB2) was affected by interaction of conditioning temperature and conditioning time. Because rumen undegradable protein (RUP) is mainly contributed by PB2 and unavailable protein (PC), it is necessary to look at *in situ* data to determine changes in protein degradation induced by pelleting.

With respect to effects of temperature and time of conditioning on the *in situ* rumen degradation characteristics of canola meal, when conditioning temperature was set at 90ºC, the pelleted samples were highest in rumen bypass neutral detergent fiber (BNDF) and rumen undegradable protein (RUP^{NRC}) ($P<0.05$). Therefore, rumen degradation of CP and NDF was

lowest at 90ºC. Moreover, higher soluble fraction (S) of CP and NDF was induced with a longer conditioning time ($P<0.05$). However, we found pellets had higher degradation rate (Kd) of CP and effectively degraded of crude protein (EDCP) ($P<0.01$) but lower RUP^{NRC} than the unprocessed canola meal ($P<0.001$). Therefore, the degradation of CP was increased after pelleting with low temperature and short processing time. Protein bypassed to the small intestine was reduced. Increased protein degradation would increase N supply in the rumen. However, when energy supply is limited, parts of N cannot get absorbed and thus used in microbial protein synthesis. Therefore, the extra N in the rumen is the potential N loss, which will increase ammonia content in the rumen. This is supported by the study of hourly effective rumen degradation ratios in Chapter 3. Ratio of effectively degraded N to effectively degraded CHO (ED_N/ED_CHO) and the ratio of effectively degraded N to effectively degraded OM (ED_N/ED_OM) in all samples were higher than optimal values. Samples conditioned at 90ºC tended to have lowest ED_N/ED_CHO and ED_N/ED_OM compared with those conditioned at 70ºC and 80ºC. This meant increasing conditioning temperature tended to reduce the potential N loss of canola meal pellets in the rumen. However, pellets had higher the ED_N/ED_CHO and ED_N/ED_OM than the unprocessed mash ($P<0.05$). Therefore, the pelleting with low temperature improved ruminal protein degradation and then intensified the potential N loss of canola meal in the rumen. At the same time, because of increased protein degradation in the rumen by pelleting, the amount of protein passed to the small intestine was decreased, and then intestinal digestible protein (IDP) was decreased. This was supported by the study of intestinal digestion that the unprocessed mash exhibited higher IDP content than pellets ($P<0.01$). Altering pelleting conditions did not affect intestinal digestibility of protein. However, increasing conditioning temperature tended to increase intestinal digestible protein (IDP) content of canola meal pellets ($P<0.10$). It can be summarized that though pelleting in this study increased rumen degradation of protein and reduced protein passed to the small intestine, but total digestible protein (TDP) was not affected.

The prediction of protein supply based on the NRC 2001 model indicated that the unprocessed mash had higher the metabolizable protein (MP^{NRC}) value of canola meal than pellets. Therefore, pelleting with low temperature reduced the protein supply of canola meal to dairy cattle. However, conditioning temperature tended to have a significant effect on MP^{NRC} ($P<0.10$). The MP^{NRC} value in pellets tended to increase with increasing conditioning temperature. The degraded protein balance in the NRC 2001 model (OEB^{NRC}) values of

canola meal pellets tended to decrease with increasing conditioning temperature ($P<0.10$). In addition, OEB^{NRC} value of canola meal was increased after pelleting with low temperature in this study ($P<0.05$). A higher OEB value means a severer level of N loss. Therefore, increasing conditioning temperature tended to reduce potential N loss of canola meal pellets, though pelleting with low temperature intensified the N loss level of canola meal. The comparison between the unprocessed mash and pellets in regards to milk production, based on data from the NRC 2001 model, revealed that pelleting with low conditioning temperature in our study slightly reduced FMV of canola meal, while samples conditioned at 90ºC were numerically highest in FMV of canola meal pellets.

In agreement with the previous study, pelleting shifted the digestion site of protein from the small intestine to the rumen (Goelema, 1999). This is related to particle size reduction or changes in protein molecular structure after pelleting (Goelema, 1999). In Chapter 4, ATR-FTIR, along with univariate and multivariate analyses, was used to determine changes of protein and carbohydrate molecular structures induced by pelleting process. There were significant differences in protein spectral profiles among the samples conditioned at different temperatures and time. Our study found that area and height of protein amide II were significantly affected by conditioning temperature ($P<0.05$) and time ($P<0.05$). Area and height of amide II were decreased with increasing conditioning temperature or decreasing conditioning time. The area and height ratios of amide I to amide II were increased with increasing conditioning temperature ($P<0.05$) or decreasing conditioning time ($P<0.05$). Therefore, different conditions during pelleting process may induce differences in protein's inherent structures. Differences between the unprocessed mash and pelleted samples in spectral profiles of protein and carbohydrates revealed that the pelleting process affected molecular structures of protein and carbohydrates. However, results from multivariate analysis based on original spectrum reported that structural make-up of protein and carbohydrate among samples were not distinguishable because of mixed clusters in CLA and overlapping ellipses in PCA. Univariate analysis only used absorption intensities of peak area and height of functional groups. However, multivariate analysis, which is based on statistical variable reduction, assessed entire information of the whole spectral region. The changes in protein and carbohydrate inherent structures caused by pelleting under different conditioning temperatures and time were not sufficient to be picked up by CLA and PCA from original spectrum. In the future, the 2^{nd} Derivative Function or the Fourier Self Deconvolution (FSD) method could be used to further process original spectra and detect the

changes which were not obvious on the original spectra. In Chapter 5, relationships between protein spectral profiles and protein profiles, rumen degradation and intestinal digestion characteristics of protein, and predicted protein supply of pelleted canola meal were also investigated. It was reported that protein spectral profiles were correlated with protein profiles, rumen degradation and intestinal digestion characteristics of protein and predicted protein supply. Hence, differences in the inherent structural make-up of protein induced by different temperatures and time of conditioning would partially explain differences in protein profiles, rumen degradation and intestinal digestion characteristics, and protein supply among samples. For example, ratio of amide I height to amide II height was positively correlated with SCP content and endogenous protein (ECP), but negatively correlated with degradable fraction (D) of CP. Because of natural characteristics of plant tissues, it is impossible to ignore the interference of protein and carbohydrates during digestion. Hence, it is necessary to investigate relationships between carbohydrate molecular structures and protein molecular structures in determining protein profiles, rumen degradation and intestinal digestion characteristics, and predicted protein supply of canola meal. The multi-regression study was carried out to select the most important structural spectral profiles of protein and carbohydrates to determine protein utilization and availability of canola meal. In addition, predicting equations based on protein and carbohydrate spectral parameters were performed. The results indicated that protein and carbohydrate spectral profiles can be used as predictors to estimate protein profiles, rumen degradation and intestinal digestion characteristics and predicted protein supply of canola meal. For example, in NRC-2001 model, one of most important equations was the MP^{NRC} = -17.03+376.61 × total carbohydrates peak 2 height + 151.14 × total carbohydrates peak 1 area (R^2 = 0.96). Moreover, both protein inherent structures and carbohydrate inherent structures play roles in determining protein profiles, rumen degradation and intestinal digestion characteristics and predicted protein supply of canola meal. For example, moderately degradable true protein (PB1) can be predicted as: 71.80 - 547.76 × structural carbohydrates peak 2 height +1.80 × total structural carbohydrate area - 5.32× ratio of amide I to amide II height (R^2 = 0.9996).

In conclusion, different temperatures and time of conditioning during the pelleting process had significant effects on nutrient profiles, rumen degradation and intestinal digestion characteristics, and predicted nutrient supply of canola meal. Increasing conditioning temperature tended to decrease protein degradation in the rumen, and increase intestinal digestible protein of canola meal pellets. Therefore, increasing conditioning temperature

tended to increase predicted protein supply and reduce potential N loss of canola meal pellets. However, pelleting with low temperature altered protein degradation and hourly effectively degradation ratios of canola meal in the rumen, and shifted the protein digestion site to the rumen. Therefore, pelleting reduced truly absorbed protein in the small intestine, and then reduced the protein supply to dairy cattle. However, intestinal digestibility of protein and total digestible protein content were not significantly affected. The ATR-FTIR detects the inherent structural changes of canola meal caused by different conditioning conditions. Protein spectral profiles were correlated with protein profiles and availability. Moreover, protein and carbohydrate spectral profiles can be used as predictors to evaluate protein profiles, digestion characteristics and predicted protein supply of pelleted canola meal. However, extensive studies with various sources of canola meal and different processing conditions are required to determine the applicability of selected parameters and estimated equations.

This study will provide benefits to the feed industry and animal nutrition researchers. Pelleted canola meal had the lowest rumen degradation when the conditioning temperature was 90ºC. Similar to the application of near infrared reflectance spectroscopy (NIR) for rapid feed evaluation on site, ATR-FTIR could also be used as an effective tool for rapidly investigating chemical composition of feed on site without applying complicated, time consuming and expensive analytical procedures. In addition, ATR-FTIR detects absorption intensities of important functional groups of protein and carbohydrates without destroying inherent structure. For example, ATR-FTIR could be used to determine effects of feed processing on protein inherent structure, and thereby assess feed processing-induced changes in protein availability of feeds for animals.

For animal nutrition researchers, increased rumen degradation of protein by pelleting is not expected. Ruminant nutritionists aim to provide animals with feeds that have sufficient N for microbial growth and protein synthesis in the rumen and increase rumen bypass protein to the small intestine of feeds. Our study revealed that reduced rumen degradation of protein induced by increasing conditioning temperature decreased potential N loss, increased protein intestinal digestion, and increased protein supply to dairy cattle of canola meal pellets. However, increased protein degradation, reduced intestinal digestion, and decreased protein supply were observed when unprocessed canola meal compared with pelleted canola meal conditioned at low temperature. This should therefore be considered in accurate diet

formulation. Though resultant nutritive feedback demonstrated that pelleting canola meal under current conditions may not provide benefits to ruminants, other advantages such as reduced selective consuming and improved feed intake are beneficial to monogastrics. The ATR-FTIR has a great application potential in feed science. This is because that ATR-FTIR can handle different kinds of feeds. In addition, the ATR-FTIR can be used in evaluating feeds without using traditional wet chemistry. In addition, ATR-FTIR can be used in fast determination of difference in protein and carbohydrates among feeds.

My study found that pelleting increased rumen degradation of protein and reduced MP^{NRC} and feed milk value (FMV) of canola meal, which was not beneficial for dairy production. Providing sufficient RUP from feeds to dairy cattle to support high level of milk production is very important in diet formulation. Pelleting with relative low temperature in my study decreased RUP rather than increased it. Therefore, studying the effects of pelleting with higher conditioning temperatures on protein profiles, rumen degradation and digestion characteristics of protein, and protein supply of canola meal to figure out whether higher conditioning temperature during pelleting have benefits for dairy production is necessary. Multi-regression analyses in our study found that carbohydrate spectral profiles can also be used as predictors in estimating equations to determine protein profiles, protein digestion characteristics, and protein supply of canola meal. Therefore, an in-depth study of the interrelationships between protein and carbohydrates on a molecular basis can help reveal how the interference of protein and carbohydrate matrices affecting digestion characteristics and availability of protein. Determining the applicability of predicted models in field is necessary.

7. Literature Cited

Abdollahi, M. R., Ravindran, V. and Svihus, B. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Anim. Feed Sci. Technol. 179 (1-4): 1-23.

Abeysekara, S., Samadi and Yu, P. 2012. Response and sensitivity of lipid related molecular structure to wet and dry heating in Canola tissue. Spectrochim. Acta Mol. Biomol. Spectros. 90: 63-71.

Abeysekara, S., Christensen, D. A., Niu, Z., Theodoridou, K. and Yu, P. 2013. Molecular structure, chemical and nutrient profiles, and metabolic characteristics of the proteins and energy in new cool-season corn varieties harvested as fresh forage for dairy cattle. J. Dairy Sci. 96 (10): 6631-6643.

American Society of Agricultural Engineers (ASAE). 1997. ASAE S269.4, Cubes, pellets and crumbles-definitions and methods for determining density, durability and moisture. Standards 1997. American Society of Agricultural Engineers, St. Joseph, MI, US.

Amerah, A. M., Ravindran, V. and Lentle, R. G. 2007. Feed particle size: implications on the digestion and performance in poultry. World Poult. Sci. J. 63: 439-451.

Arntfield, S. and Hickling, D. 2011. Meal nutrition and utilization. In J.K. Daun, N.A.M. Eskin, and D. Hickling (eds.), Canola Chemistry, Production, Processing, and Utilization. AOCS Press, Urbana, IL, US. pp 281-304.

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, US.

Barthet, V. J. and Daun, J. K. 2011. Seed morphology, composition, and quality. In: J.K. Daun, N.A.M. Eskin, D. Hickling (eds.), Canola Chemistry, Production, Processing, and Utilization. AOCS Press, Urbana, IL, US. pp 119-154.

Behnke, K. C. 1994. Factors affecting pellet quality. Proc. Maryland Nutrition Conference. Dept. of Poultry Science and Animal Science, College of Agriculture, University of Maryland, College Park, MD, US.

Belanche, A., Weisbjerg, M. R., Allison, G. G., Newbold, C. J., and Moorby, J. M. (2013). Estimation of feed crude protein concentration and rumen degradability by Fourier-transform infrared spectroscopy. J. Dairy Sci. 96 (12): 7867-7880.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73: 679-697.

Boila, R. J. and Ingalls, J. R. 1992. In situ rumen digestion and escape of dry matter, nitrogen and amino acids in canola meal. Can. J. Anim. Sci. 72: 891-901.

Brito, A. F. and Broderick, G. A. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. J. Dairy Sci. 90: 1816-1827.

Büchl, N. R., Hutzler, M., Mietke-Hofmann, H., Wenning, M., & Scherer, S. (2010). Differentiation of probiotic and environmental Saccharomyces cerevisiae strains in animal feed. J. Appl. Microbiol. 109 (3): 783-791.

Calsamiglia, S. and Stern, M. D. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73: 1459-1465.

Camire, M. E., Camire, A. and Krumhar, K. 1990. Chemical and nutritional changes in food during extrusion. Crit. Rev. Food Sci. Nutr. 29: 35-57.

Canadian council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals (2nd ed). Canadian Council of Animal Care. Ottawa, ON, Canada.

Canola Council of Canada. 2013. The Economic Impact of Canola on the Canadian Economy. Accessed on May 2014: http://www.canolacouncil.org/media/545722/lmc_economic_impact_of_canola_on_the_canadian_economy_october_2013.pdf.

Cramer, K. R., Wilson, K. J., Moritz, J. S. and Beyer, R. S. 2003. Effect of sorghum-based diets subjected to various manufacturing procedures on broiler performance. J. Appl. Poult. Res. 12: 404-410.

Czerkawski, J. W. 1986. An Introduction to Rumen Studies. Pergamon Press, Oxford, NY. US.

Dhanoa, M. S. 1988. On the analysis of dacron bag data for low degradability feeds. Grass and Forage Sci. 43: 441-444.

Doiron, K. J., Yu, P., Christensen, C. R., Christensen, D. A. and McKinnon, J. J. 2009. Detecting molecular changes in Vimy flaxseed protein structure using synchrotron FTIRM and DRIFT spectroscopic techniques: Structural and biochemical characterization. J. Spectro. 23 (5-6): 307-322.

Ebrahimi, S. R., Nikkhah, A. and Sadeghi, A. A. 2010. Changes in nutritive value and digestion kinetics of canola seed due to microwave irradiation. Asian-Aust. J. Anim. Sci. 23: 347-354.

Elmore, D. L., Lendon, C. A., Smith, S. A. and Leverette, C. L. 2005. Mid-infrared imaging applications in agricultural and food sciences. In: R. Bhargava and I. W. Levin (eds.), Spectrochemical Analysis Using Infrared Multichannel Detectors. Wiley-Blackwell, Oxford, UK. pp 261-282.

Eskin, N. A. M. 2013. Canola research: historical and recent aspects. In: U. Thiyam-Holländer, N.A.M. Eskin, B. Matthäus et al. (eds.), Canola and Rapeseed: Production, Processing, Food Quality and Nutrition. CRC Press, Boca Raton, FL, US. pp 1-19.

Fairchild, F. and Greer, D. 1999. Pelleting with precise mixer moisture control. Feed Int. 20 (8): 32-36.

Fairfield, D., Thomas, H., Garrison, R., Bliss, J., Behnke, K. and Gilpin, A. 2005. In E. K. Schofield (ed), Feed Manufacturing Technology V. American Feed Manufacturers Association, Arlington, VA, US. pp 142-167.

Falk, D. 1985. Pelleting cost centre. In: R. R. Mcellhiney, (Ed.), Feed Manufacturing Technology III. American Feed Manufacturers Association, Arlington, VA, US. pp 167-190.

Fox, D. G., Tedeschi, L. O., Tylutki, T. P., Russel, J. B., Van Amburgh M. E., Chase, L. E., Pell, A. N. and Overton, T. R. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112: 29-78.

Gamage, I. H., Jonker, A., Christensen, D. A. and Yu, P. 2012. Metabolic characteristics of proteins and biomolecular spectroscopic profiles in different batches of feedstock (wheat) and their co-products (wheat dried distillers grains with solubles) from the same bioethanol processing plant. J. Dairy Sci. 95: 1-21.

Gargallo, S., Calsamiglia, S. and Ferret, A. 2006. Technical note: a modified three-step in vitro procedure to determine intestinal digestion of proteins. J. Anim. Sci. 84 (8): 2163-2167.

Goelema, J. O. 1999. Processing of legume seeds: effects on digestive behaviour in dairy cows. Ph.D. Thesis. Wageningen University, Wageningen, Netherlands.

Harstad, O. M. and Prestløkken, E. 2001. Rumen degradability and intestinal indigestibility of individual amino acids in corn gluten meal, canola meal and fish meal determined *in situ*. Anim. Feed Sci. Technol. 94: 127-135.

Heendeniya, R. G. 2008. Utilization of canola seed fractions in ruminant feeds. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK, Canada.

Heendeniya, R. G., Christensen, D. A., Maenz, D. D., McKinnon, J. J. and Yu, P. 2012. Protein fractionation byproduct from canola meal for dairy cattle. J. Dairy Sci. 95 (8): 4488-4500.

Higgs, R. J., Chase, L. E., Ross, D. A. and Van Amburgh, M. E. 2012. Evaluating and refining the CNCPS feed library using commercial laboratory feed databases. Accessed May 2014: http://www.ansci.cornell.edu/pdfs/cnc2012_Higgs.txt.pdf

Huang, A. H. C. 1992. Oil bodies and oleosins in seeds. Ann. Rev. Plant Physiol. Plant Molec. Biol. 43: 177-200.

Jackson, M. and Mantsch, H. H. 1995. The use and misuse of FTIR spectroscopy in the determination of protein structure. Crit. Rev. Biochem. Mol. Biol. 30: 95-120.

Jackson, M. and Mantsch, H. H. 2000. Infrared spectroscopy ex vivo tissue analysis. In: R. A. Meyers (ed.), Encyclopedia of Analytical Chemistry. Wiley, Chichester, UK. pp 131-156.

Jonker, A., Gruber, M. Y., Wang, Y., Coulman, B., McKinnon, J. J., Christensen, D. A. and Yu, P. 2012. Foam stability of leaves from anthocyanidin-accumulating Lc-alfalfa and relation to molecular structures detected by FTIR vibration spectroscopy. Grass Forage Sci. 67: 369-381.

Kinsella, J. E. 1979. Functional properties of soy proteins. J. Am. Oil Chem. Sot. 56: 242-258.

Lanzas, C., Broderick, G. A. and Fox, D. G. 2008. Improved feed protein fractionation schemes for formulating rations with the Cornell Net Carbohydrate and Protein System. J. Dairy Sci. 91 (12): 4881-4891.

Lanzas, C., Sniffen, C. J., Seo, S., Tedeschi, L. O. and Fox, D. G. 2007. A feed carbohydrate fractionation scheme for formulating rations for ruminants. Anim. Feed Sci. Technol. 136: 167-190.

Licitra, G., Hernandez, T. M. and van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Sci. Feed Technol. 57: 347-358.

Liu, B., McKinnon, J. J., Thacker, P. and Yu, P. 2012. Molecular structure and metabolic characteristics of the proteins and energy in triticale grains and dried distillers grains with solubles for dairy cattle. J. Agric. Food Chem. 60: 10064-10074.

Liu, N. 2009. Ruminal nutrient availability and inherent structural features of six barley varieties using *in situ* technique and mid-IR spectroscopy. M.Sc. Thesis. University of Saskatchewan, Saskatoon, SK, Canada

Liu, N. and Yu, P. 2011. Molecular clustering, interrelationships and carbohydrate conformation in hull and seeds among barley cultivars. J. Cereal. Sci. 53 (3): 379-383.

Maier, D. E. and Bakker-Arkema, F. W. 1992. The counterflow cooling of feed pellets. J. Agric. Eng. Res. 53: 305-319.

Mahesar, S. A., Sherazi, S. T. H., Kandhro, A. A., Bhanger, M. I., Khaskhel, A. R. I and Talpur, M. Y. 2011.Evaluation of important fatty acid ratios in poultry feed lipids by ATR FTIR spectroscopy, Vib. Spectrosc. 57 (2): 177-181.

Martin, A. A., Carter, R. A. B., de Oliveira Nunes, L., Arisawa, E. A. L. and Silveira Jr, L. 2004. Principal components analysis of FT-Raman spectra of ex vivo basal cell carcinoma. In: Z. Gryczynski, T. Vo-Dinh, J. R. Lakowicz, A. Mahadevan-Jansen, M. G. Sowa, and G. J. Puppels (Eds.), Biomedical Vibrational Spectroscopy and Biohazard Detection Technologies. 5321. SPIE, San Jose, CA. pp 198-204.

McKinnon, J., Landner, H., Penner, G. and McAllister, T. 2014. Evaluation of canola meal as a protein and energy source for cattle: final report. Saskatchewan Agriculture Development Fund Project 2010-176. Accessed May 2014: http://library2.usask.ca/gp/sk/da/adf/20100176.pdf

McKinnon, J. J., Olubobokun, J. A., Mustafa, A., Cohen, R. D. H. and Christensen, D. A. 1995. Influence of dry heat treatment of canola meal on site and extent of nutrient disappearance in ruminants. Anim. Feed Sci. Technol. 56 (3): 243-252.

Moritz, J. S. and Lilly, K. G. S. 2010. Production strategies and feeding opportunities for pellets of high quality. In: Proceedings of the 8th Annual Mid-Atlantic Nutrition Conference, University of Maryland, College Park, MD, US. pp 85-90.

Murphy, D. J. 1993. Structure, function and biogenesis of storage lipid bodies and oleosins in plants. Prog. Lipid Res. 32: 247-280.

Mustafa, A. F., Christensen, D. A., McKinnon, J. J. and Newkirk, R. 2000. Effects of stage of processing of canola seed on chemical composition and in vitro protein degradability of canola meal and intermediate products. Can. J. Anim. Sci. 80 (1): 211-214.

Mustafa, A. F., Gonthier, C. and Ouellet, D. R. 2003a. Effects of extrusion of flaxseed on ruminal and postruminal nutrient digestibilities. Arch. Anim. Nutr. 57: 455-463.

Mustafa, A. F., Chouinard, Y. P., Ouellet, D. R. and Soita, H. 2003b. Effects of moist heat treatment on ruminal nutrient degradability of sunflower seed. J. Sci. Food Agric. 83: 1059-1064.

Newkirk, R. 2009. Canola meal feed industry guide: 4th Edition. Accessed on May 2014: http://www.canolacouncil.org/media/516716/canola_meal_feed_guide_english.pdf.

Newkirk, R. 2011. Meal nutrient composition. In: J.K. Daun, N.A.M. Eskin, and D. Hickling (eds.), Canola Chemistry, Production, Processing, and Utilization. AOCS Press, Urbana, IL, US. pp229-242.

Newkirk, R. W., Classen, H. L., Scott, T. A. and Edney, M. J. 2003. The digestibility and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. 83:131-139.

Nia, S. M., and Ingalls, J. R. 1992. Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. Can. J. Anim. Sci., 72 (1): 83-88.

Nietner, T., Pfister, M., Brakowiecka-Sassy, B., Glomb, M. A. and Fauhl-Hassek, C. 2015. Screening for Sulfate in Distillers Dried Grains and Solubles by FT–IR Spectroscopy. J. Agric. Food Chem. 63(2): 476-484.

Nocek, J. E. 1988. In situ and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci. 71: 2051-2069.

Nolan, A., McDonnell, K., Devlin, G. J., Carroll, J. P. and Finnan, J. 2010. Economic analysis of manufacturing costs of pellet production in the Republic of Ireland using non-woody biomass. Open Renew. Energy J. 3: 1-11.

Novak, C., Yakout, H. and Scheideler, S. 2004. The combined effects of dietary lysine and total sulfur amino acid level on egg production parameters and egg components in Dekalb Delta laying hens. Poult. Sci. 83: 977-984.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th ed. National Research Council, National Academy of Science, Washington, DC, US.

NRC. 2001. Nutrient Requirement of Dairy cattle. 7th ed. National Research Council, National Academy of Science, Washington, DC, US.

Nuez-Ortín, W. G. and Yu, P. 2010. Estimation of ruminal and intestinal digestion profiles, hourly effective degradation ratio and potential N to energy synchronization of co-products from bioethanol processing. J. Sci. Food Agric. 90: 2058-2067.

Ørskov, E. R., DeB Hovell, F. D. and Mould, F. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5: 195-213.

Ørskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92: 499-503.

Pasangulapati, V., Ramachandriya, K. D., Kumar, A., Wilkins, M. R., Jones, C. L. and Huhnke, R. L. 2012. Effects of cellulose, hemicellulose and lignin on thermochemical conversion characteristics of the selected biomass. Bioresource. Technol. 114: 663-669.

Peng, Q., Wang, Z., Zhang, X. and Yu, P. 2014a. Common prairie feeds with different soluble and insoluble fractions used for CPM diet formulation in dairy cattle: Impact of carbohydrate-protein matrix structure on protein and other primary nutrient digestion. Spectrochim. Acta Mol. Biomol. Spectros. 121: 14-22.

Peng, Q., Khan, N. A., Wang, Z. and Yu, P. 2014b. Moist and dry heating-induced changes in protein molecular structure, protein subfractions, and nutrient profiles in camelina seeds. J. Dairy Sci. 97 (1): 446-457.

Piepenbrink, M. S. and Schingoethe, D. J. 1998. Ruminal degradation, amino acid composition and estimated intestinal digestibilities of four protein supplements. J. Dairy Sci. 81: 454-461.

Plaisance, R., Petit, H. V., Seoane, J. R. and Rioux, R. 1997. The nutritive value of canola, heat-treated canola and fish meals as protein supplements for lambs fed grass silage. J. Anim. Feed Sci. Technol. 68 (1): 139-152.

Rahman, M. H., Joersbo, M., and Poulsen, M. H. 2001. Development of yellow-seeded *Brassica napus* of double low quality. Plant Breeding. 120 (6): 473-478.

Rao, M. A. and Lund, D. B. 1986. Kinetics of thermal softening of foods-a review. J. Food Process Preserv. 10: 31l-329.

Rashid, A., Rakow, G. and Downey, R. K. 1994. Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breeding. 112 (2): 127-134.

Robinson, P. H., Fadel, J. G. and Tamminga, S. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim. Feed Sci. Technol. 15: 249-271.

Roe, M. B., Sniffen, C. J. and Chase, L. E. 1990. Techniques for measuring protein fractions in feedstuffs, In: Proc Cornell Nutrition Conference. Department of Animal Science, Cornell University, Ithaca, NY, US. pp 81-88.

Sadeghi, A. A., and Shawrang, P. 2006. Effects of microwave irradiation on ruminal degradability and *in vitro* digestibility of canola meal. J. Anim. Feed Sci. Technol. 127(1-2): 45-54.

Samadi, S. and Yu, P. 2011. Dry and moist heating-induced changes in protein molecular structure, protein subfraction, and nutrient profiles in soybeans. J. Dairy Sci. 94: 6092-6102.

Samadi, Theodoridou, K. and Yu, P. 2013. Detect the sensitivity and response of protein molecular structure of whole canola seed (yellow and brown) to different heat processing methods and relation to protein utilization and availability using ATR-FT/IR molecular spectroscopy with chemometrics. Spectrochim. Acta Mol. Biomol. Spectros. 105: 304-310.

Simbaya, J., Slominski, B. A., Rakow, G., Campbell, L. D., Downey, R. K. and Bell, J. M. 1995. Quality characteristics of yellow-seeded Brassica seed meals: Protein, carbohydrate, and dietary fiber components. J. Agric. Food Chem. 43 (8): 2062-2066.

Sinclair, L. A., Garnsworthy, P. C., Bearijsworth, P., Freeman, P. and Buttery, P. J. 1991. The use of cytosine as a marker to estimate microbial protein synthesis in the rumen. Anim. Prod. 52: 592 (Abstr).

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R. and Buttery, P. J. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 120: 251-263.

Skoch, E. R., Behnke, K. C., Deyoe, C. W. and Binder, S. F. 1981. The effect of steam-conditioning rate on the pelleting process. Anim. Feed Sci. Technol. 6: 83-90.

Skoch, E. R., Binder, S. F., Deyoe, C. W., Allee, G. L. and Behnke, K. C. 1983. Effects of pelleting conditions on performance of pigs fed a corn-soybean meal diet. J. Anim. Sci. 57: 922-928.

Schingoethe, D.J. 1991. Protein quality, amino acid supplementation in dairy cattle explored. Feedstuffs.March18, 1991. pp 11.

Smith, B. C. 2011. Fundamentals of Fourier transform infrared spectroscopy. CRC Press. Boca Raton, FL, US.

Spörndly, E. and Åsberg, T. 2006. Eating rate and preference of different concentrate components for cattle. J. Dairy Sci. 89: 2188-2199.

Stringam, G. R., Mcgregor, D. I. and Pawlowski, S. H. 1974: Chemical and morphological characteristics associated with seed coat colour in rapeseed. Proc.4th Int. Rapeseed Cong. GiefSen, Germany. pp 99-108.

Stuart, B. H. 2004. Infrared Spectroscopy: Fundamentals and Applications. John Wiley and Sons, Chichester, West Sussex, UK.

Tamminga, S. and Goelema, J. O. 1995. The significance of rate and site of starch digestion in ruminants. Carbohydrates in feeds for ruminants. Proc. SCI, London, UK.

Tamminga, S., Van Straalen, W. M., Subnel, A. P. J., Meijer, R. G. M., Steg, A., Wever, C. J. G. and Blok, M. C. 1994. The Dutch protein evaluation system: The DVE/OEB-system. Lives. Prod. Sci. 40: 139-155.

Tamminga, S., van Vuuren, A. M., van der Koelen, C. J., Ketelaar, R. S. and van der Togt, P. L. 1990. Ruminal behavior of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Neth. J. Agric. Sci. 38: 513-526.

Theodoridou, K. and Yu, P. 2013a. Application potential of ATR-FT/IR molecular spectroscopy in animal nutrition: revelation of protein molecular structures of canola meal and presscake, as affected by heat-processing methods, in relationship with their protein digestive behavior and utilization for dairy cattle. J. Agric. Food Chem. 61 (23): 5449-5458.

Theodoridou, K. and Yu, P. 2013b. Metabolic characteristics of the proteins in yellow-seeded and brown-seeded canola meal and presscake in dairy cattle: comparison of three systems (PDI, DVE, and NRC) in nutrient supply and feed milk value (FMV). J. Agric Food Chem. 61 (11): 2820-2830.

Theodoridou, K. and Yu, P. 2013c. Effect of processing conditions on the nutritive value of canola meal and presscake. Comparison of the yellow and brown-seeded canola meal with the brown-seeded canola presscake: Effect of processing conditions on the nutritive value of canola meal and canola presscake. J. Sci. Food Agric. 93 (8): 1986-1995.

Thomas, M., van Vliet, T. and van der Poel, A. F. B. 1998. Physical quality of pelleted animal feed 3. Contribution of feedstuff components. Anim. Feed Sci. Technol. 70 (1): 59-78.

Thomas, M. and van der Poel, A. F. B. 1996. Physical quality of pelleted animal feed 1. Criteria for pellet quality. Anim. Feed Sci. Technol. 61 (1): 89-112.

Thomas, M., Van Zuilichem, D. J. and Van der Poel, A. F. B. 1997. Physical quality of pelleted animal feed. 2. Contribution of processes and its conditions. Anim. Feed Sci. Technol. 64 (2): 173-192.

Tylutki, T. P., Fox, D. G., Durbal, V. M., Tedeschi, L. O., Russell, J. B., Van Amburgh, M. E. and Pell, A. N. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Anim. Feed Sci. Technol. 143 (1-4): 174-202.

Tyrrell, H. F. and Moe, P. W. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58: 1151-1163.

Unger, E. H. 2011. Processing. In: J.K. Daun, N.A.M. Eskin, and D. Hickling (eds.), Canola Chemistry, Production, Processing, and Utilization. AOCS Press, Urbana, IL, US. pp 163-188.

Van Amburgh, M. E., Chase, L. E., Overton, T. R., Ross, D. A., Recktenwald, E. B., Higgs, R. J. and Tylutki, T. P. 2010. Updates to the Cornell Net Carbohydrate and Protein System v6. 1 and implications for ration formulation. In Proc. Cornell Nutrition Conf. for Feed Manufacturers. Accessed on May 2014: http://ansci1.abc.cornell.edu/cnconf/2010proceedings/CNC2010.17.VanAmburgh.pdf

Van Amburgh, M. E., Foskolos, A., Collao-Saenz, E. A., Higgs, R. J. and Ross, D. A. 2013. Updating the CNCPS feed library with new feed amino acid profiles and efficiencies of use: evaluation of model predictions-version. Accessed on May 2014: http://www.ansci.cornell.edu/pdfs/CNC2013_VanAmburgh_m.pdf.

Van Soest, P. 1989. On the digestibility of bound N in distillers grains: A reanalysis. Proc. Cornell Nutr. Conf. October 24-26, 1989. Syracuse, NY, US. pp 127-136.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Van der Poel, A. F. B., Verstegen, M. W. A. and Tarnminga, S. 1995. Chemical, physical and nutritional effects of feed processing technology. In: 16th Western Nutrition Conference, 13- 14 September 1995, Saskatoon, SK, Canada. pp 266.

Voragen, A. G. J., Gruppen, H., Marsman, G. J. P. and Mul, A. J. 1995. Effect of some manufacturing technologies on chemical, physical and nutritional properties of feed. In: P.C. Garnsworthy and D.J.A. Cole. (eds.), Recent Advances in Animal Nutrition, University of Nottingham Feed Manufacturers Conference 1995. Nottingham University Press. pp 93-126.

Weiss, W. P. 1998. Estimating the available energy content of feeds for dairy cattle. J. Dairy Sci. 81: 830-839.

Weiss, W. P., Conrad, H. R. and St. Pierre, N. R. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol. 39: 95-110.

Wetzel, D. L., Eilert, A. J., Pietrzak, L. N., Miller, S. S. and Sweat, J. A. 1998. Ultraspatially resolved synchrotron infrared microspectroscopyof plant tissue in situ. Cell. Mol. Biol. 44: 145-168.

Xin, H. and Yu, P. 2013a. Using ATR-FT/IR to detect carbohydrate-related molecular structure features of carinata meal and their in situ residues of ruminal fermentation in comparison with canola meal. Spectrochim. Acta Mol. Biomol. Spectros. 114: 599-606.

Xin, H. and Yu, P. 2013b. Detect changes in protein structure of carinata meal during rumen fermentation in relation to basic chemical profile and comparison with canola meal using ATR-FT/IR molecular spectroscopy with chemometrics. Spectrochim. Acta Mol. Biomol. Spectros. 112: 318-325.

Xin, H. and Yu, P. 2013c. Chemical profile, energy values, and protein molecular structure characteristics of biofuel/bio-oil co-products (carinata meal) in comparison with canola meal. J. Agric. Food Chem. 61 (16): 3926-3933.

Yan, X., Khan, N. A., Zhang, F., Yang, L. and Yu, P. 2014. Microwave irradiation induced changes in protein molecular structures of barley grains: relationship to changes in protein chemical profile, protein subfractions, and digestion in dairy cows. J. Agric. Food Chem. 62 (28): 6546-6555.

Yang, L. 2012. Effect of carbohydrate traits on nutritional characteristics and spectral features of molecular structure in hulless barley. M.Sc Thesis. University of Saskatchewan, Saskatoon. SK, CA.

Yang, L., Christensen, D. A., McKinnon, J. J., Beattie, A. D. and Yu, P. 2013a. Effect of altered carbohydrate traits in hulless barley (Hordeum vulgare L.) on nutrient profiles and availability and nitrogen to energy synchronization. J. Cereal Sci. 58 (1):182-190.

Yang, L., Christensen, D. A., McKinnon, J. J., Beattie, A. D., Xin, H. and Yu, P. 2013b. Investigating the molecular structural features of Hulless Barley (Hordeum vulgare L.) in relation to metabolic characteristics using Synchrotron-Based Fourier Transform Infrared Microspectroscopy. J. Agric. Food Chem. 61 (47): 11250-11260.

Yang, L., McKinnon, J. J., Christensen, D. A., Beattie, A. D. and Yu, P. 2014. Characterizing the molecular structure features of newly developed hulless barley cultivars with altered carbohydrate traits (Hordeum vulgare L.) by globar-sourced infrared spectroscopy in relation to nutrient utilization and availability. J. Cereal. Sci. 60 (1): 48-59.

Yu, P., Goelema, J. O. and Tamminga, S. 2000. Using the DVE/OEB model todetermine optimal conditions of pressure toasting on horse beans (Vicia faba) for the dairy feed industry. Anim. Feed Sci. Technol. 86: 165-176.

Yu, P., Goelema, J. O., Leury, B. J., Tamminga, S. and Egan, A. R. 2002. An analysis of the nutritive value of heat processed legume seeds for animal production using the DVE/OEB model: a review. J. Anim. Feed Sci. Technol. 99 (1–4): 141-176.

Yu, P. 2004. Application of advanced synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy to animal nutrition and feed science: A novel approach. Br. J. Nutr. 92: 869-885.

Yu, P., McKinnon, J. J., Christensen, C. R. and Christensen, D. A. 2004. Using synchrotron transmission FTIR microspectroscopy as a rapid, direct and non-destructive analytical technique to reveal molecular microstructural-chemical featureswithin tissue in grain barley. J. Agric. Food Chem. 52: 1484-1494.

Yu, P. 2005a. Prediction of protein supply to ruminants from concentrates: comparison of the NRC-2001 model with the DVE/OEB system. J. Sci. Food Agric. 85: 527-538.

Yu, P. 2005b. Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) microspectroscopy. J. Agric. Food Chem. 53: 7115-7127.

Yu, P. 2005c. Protein secondary structures (α-helix and β-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: A new approach. Br. J. Nutr. 94: 655-665.

Yu, P. 2005d. Application of cluster analysis (CLA) in feed chemical imaging to accurately reveal structural-chemical features. J. Agric. Food Chem. 53 (8): 2872-2880.

Yu, P. 2006. An emerging method for rapid characterization of feed structures and feed component matrix at a cellular level and relation to feed quality and nutritive value. Spectroscopy. 20: 229-251.

Yu, P. 2007. Protein molecular structures, protein subfractions, and protein availability affected by heat processing: A review. Ameri. J. Biochemi. Biotechnolo. 3 (2): 66-86.

Yu, P., Doiron, K. and Liu, D. 2008. Shining light on the differences in molecular structural chemical make-up and the cause of distinct degradation behavior between malting-and feed-type barley using synchrotron FTIR microspectroscopy: A novel approach. J. Agric. Food Chem. 56 (9): 3417-3426.

Yu, P. 2008. Synchrotron−based microspectroscopic analysis of molecular and biopolymer structures using multivariate techniques and advanced multi-components modeling. Can. J. Anal. Sci. Spectrosc. 53: 220-231.

Yu, P. and Nuez-Ortín, W. G. 2010. Relationship of protein molecular structure to metabolizable proteins in different types of dried distillers grains with solubles: A novel approach. Br. J. Nutr. 104 (10):1429-1437.

Zhang, X. and Yu, P. 2012a. Using ATR-FT/IR molecular spectroscopy to detect effects of blend DDGS inclusion level on the molecular structure spectral and metabolic characteristics of the proteins in hulless barley. Spectrochim. Acta Mol. Biomol. Spectros. 95: 53-63.

Zhang, X. and Yu, P. 2012b. Differentiation of mixtures of co-product blend with barley grain based on Fourier transform infrared attenuated total reflection molecular spectroscopy: Carbohydrate molecular spectral profiles and nutritive characteristics in dairy cattle. J. Dairy. Sci. 95 (11): 6624-6634.

Zhang, X., Yan, X., Beltranena, E. and Yu, P. 2014. Molecular spectroscopic investigation on fractionation-induced changes on biomacromolecule of co-products from bioethanol processing to explore protein metabolism in ruminants. Spectrochim. Acta Mol. Biomol. Spectros. 122: 591-597.

8. Appendix

Table 8.1 Correlations between carbohydrate ATR-FTIR spectral profiles and rumen degradation and intestinal digestion characteristics of pelleting-processed canola meal

Items	Structural CHO peak 1 height	Structural CHO peak 2 height	Structural CHO peak 3 height	Structural CHO area	cellulosic height	cellulosic area	total CHO peak 1 height	Total CHO peak 2 height	Total CHO peak 3 height	Total CHO peak 1 area	Total CHO peak 2 area	Total CHO peak 3 area	Total CHO area
					Spearman Correlation R values								
Basic protein profiles													
CP (%DM)	0.06	-0.55	-0.44	-0.29	-0.58	-0.51	0.28	-0.39	-0.50	-0.66	-0.38	-0.49	-0.49
SCP (%CP)	0.01	0.49	0.33	0.17	0.37	0.30	-0.37	0.26	0.34	0.25	0.38	0.40	0.39
NDICP (%CP)	0.24	0.17	0.21	0.23	0.26	0.22	0.44	-0.16	0.19	0.32	0.11	0.13	0.14
ADICP (%CP)	0.42	0.30	0.32	0.23	0.32	0.25	0.38	-0.34	0.21	0.12	0.31	0.20	0.21
Protein subfractions													
PA2 (%CP)	0.01	0.49	0.33	0.17	0.37	0.30	-0.37	0.26	0.34	0.25	0.38	0.40	0.39
PB1 (%CP)	-0.13	-0.73+	-0.55	-0.36	-0.64	-0.54	0.25	-0.28	-0.56	-0.55	-0.55	-0.60	-0.59
PB2 (%CP)	-0.05	0.10	0.09	0.14	0.17	0.16	0.14	0.14	0.15	0.38	-0.02	0.10	0.10
PC (%CP)	0.46	0.38	0.41	0.31	0.42	0.36	0.36	-0.32	0.31	0.23	0.39	0.29	0.30
TP (%CP)	-0.47	-0.38	-0.41	-0.31	-0.43	-0.36	-0.36	0.33	-0.32	-0.23	-0.40	-0.29	-0.30
PA2_TP (%CP)	0.04	0.51	0.35	0.19	0.39	0.32	-0.35	0.24	0.36	0.27	0.40	0.42	0.41
PB1_TP (%CP)	-0.03	-0.65	-0.46	-0.30	-0.55	-0.46	0.32	-0.35	-0.50	-0.51	-0.46	-0.54	-0.53
PB2_TP (%CP)	-0.02	0.13	0.13	0.16	0.21	0.19	0.16	0.12	0.18	0.40	0.02	0.12	0.13
Estimated energy profiles													
tdCP (%DM)	0.00	-0.60	-0.50	-0.33	-0.64	-0.56	0.24	-0.36	-0.54	-0.69	-0.43	-0.53	-0.53
TDN1x, %DM	0.64	0.50	0.63	0.53	0.56	0.59	0.02	-0.51	0.53	0.35	0.63	0.52	0.53
TDN3x, %DM	0.65	0.52	0.63	0.53	0.57	0.59	0.03	-0.51	0.53	0.36	0.63	0.52	0.53
DE1x, Mcal/kg,	0.52	0.26	0.40	0.32	0.31	0.37	-0.10	-0.59	0.29	0.09	0.42	0.28	0.29
DEp3x, Mcal/kg	0.31	-0.24	-0.24	-0.09	-0.41	-0.32	0.05	-0.76	-0.30	-0.52	-0.23	-0.32	-0.33
MEp3x, Mcal/kg	0.26	-0.24	-0.05	-0.06	-0.15	-0.07	-0.07	-0.61	-0.17	-0.34	-0.01	-0.18	-0.17
NElp3x, Mcal/kg	0.25	-0.19	-0.02	-0.01	-0.07	-0.07	0.51	-0.29	-0.12	-0.20	0.01	-0.14	-0.12

Table 8.1 Cont'd.

ME, Mcal/kg,	0.75^{+}	-0.40	-0.33	-0.50	-0.19	-0.25	-0.21	0.95**	-0.30	-0.17	-0.22	-0.22	-0.22
NEm, Mcal/kg,	0.44	0.00	0.08	0.12	-0.03	-0.03	0.49	-0.44	-0.01	-0.21	0.13	-0.01	-0.07
NEg, Mcal/kg,	0.07	-0.13	0.22	0.00	0.26	0.30	-0.18	-0.13	0.09	0.04	0.27	0.09	0.11

Notes: n=14. R: Correlation coefficient calculated using spearman method; CP: crude protein; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; SCP: soluble crude protein; PA2: rapidly degradable true protein; PB1: moderately degradable true protein. PB2: slowly degradable true protein; PC: undegradable protein; TP: true protein; PA2_TP: PA2 presents in %TP basis; PB1: PB1 presents in %TP basis; PB2_TP: PB2 presents in %TP basis; tdCP: total digestible crude protein; TDN_{1x}: total digestible nutrients; TDN_{3x}: total digestible nutrients at 3x maintenance; DE_{1x}: digestible energy; DE_{p3x}: digestible energy at a production level (3x maintenance); ME_{p3x}: metabolizable energy at a production level (3x maintenance); NE_{Lp3x}: Net energy at a production level (3x maintenance); ME: metabolizable energy; NE_m: net energy for maintenance; NE_g: net energy for gain. "+": P<0.10; "*": P<0.05; "**": P<0.01.

Table 8.2 Correlations between carbohydrate ATR-FTIR spectral profiles and rumen degradation and intestinal digestion characteristics of pelleting-processed canola meal

Items	Structural CHO peak 1 height	Structural CHO peak 2 height	Structural CHO peak 3 height	Structural CHO area	cellulosic height	cellulosic area	Total CHO peak 1 height	Total CHO peak 2 height	Total CHO peak 3 height	Total CHO peak 1 area	Total CHO peak 2 area	Total CHO peak 3 area	Total CHO area
					Spearman Correlation R values								
Rumen degradation kinetics of protein													
K_d (%/h)	-0.18	-0.32	-0.55	-0.46	-0.63	-0.60	-0.43	0.44	-0.56	-0.71^{+}	-0.54	-0.54	-0.57
T_0 (h)	-0.34	-0.37	-0.60	-0.52	-0.62	-0.58	-0.60	-0.42	-0.59	-0.59	-0.68^{+}	-0.63	-0.64
S (%CP)	0.20	-0.42	-0.46	-0.20	-0.64	-0.57	0.36	-0.62	-0.49	-0.64	-0.46	-0.52	-0.52
D (%CP)	-0.10	0.40	0.48	0.25	0.66	0.59	-0.15	0.51	0.49	0.68^{+}	0.45	0.50	0.51
U (%CP)	-0.18	-0.07	-0.21	-0.18	-0.27	-0.24	-0.39	0.09	-0.17	-0.30	-0.13	-0.11	-0.13
%BCP (%CP)	0.04	0.49	0.49	0.44	0.76*	0.69^{+}	0.09	0.62	0.65	0.81*	0.62	0.67^{+}	0.68^{+}
RUP^{NRC} (g/kg, DM)	0.09	0.51	0.64	0.47	0.77*	0.07^{+}	0.15	0.59	0.67^{+}	0.82*	0.64	0.69^{+}	0.70^{+}
BCP^{DVE} (g/kg, DM)	0.09	0.51	0.64	0.47	0.77*	0.70^{+}	0.15	0.59	0.67^{+}	0.82*	0.64	0.69^{+}	0.70^{+}
%EDCP (%CP)	-0.04	-0.49	-0.62	-0.44	-0.76*	-0.69^{+}	-0.09	-0.62	-0.65	-0.81*	-0.62	-0.67^{+}	-0.68^{+}
EDCP (g/kg, DM)	0.04	-0.45	-0.57	-0.37	-0.72^{+}	-0.65	-0.01	-0.69	-0.61	-0.76*	-0.59	-0.64	-0.64
Intestinal digestion of protein													
%dIDP(%RUP)	0.42	0.65	0.79*	0.77*	0.81*	0.81*	0.25	0.29	0.85*	0.93**	0.77*	0.85*	0.85*
IDP(%RUP)	0.19	0.58	0.71^{+}	0.58	0.81*	0.77*	0.16	0.52	0.76*	0.89**	0.70^{+}	0.77*	0.77*
IDP(g/kg DM)	0.22	0.59	0.72^{+}	0.60	0.81*	0.77*	0.20	0.50	0.77*	0.90**	0.71^{+}	0.78*	0.78*
TDP(%CP)	0.54	0.10	0.05	0.34	-0.15	-0.41	0.21	-0.69^{+}	0.09	-0.06	0.01	0.05	0.04
TDP(g/kg,DM)	0.64	0.06	0.06	0.37	-0.15	-0.05	0.47	-0.79*	0.07	-0.08	0.01	0.01	0.01

Notes: n=14. R: Correlation coefficient calculated using spearman method; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T0: lag time in the rumen; S: soluble fraction in the in situ incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded crude protein; IDP: intestinal digestible protein; dIDP: intestinal digestibility of rumen undegraded protein; RUP: rumen undegraded protein; TDP: total digestible protein. “+”: P<0.10; “*”: P<0.05 “**”: P<0.01.

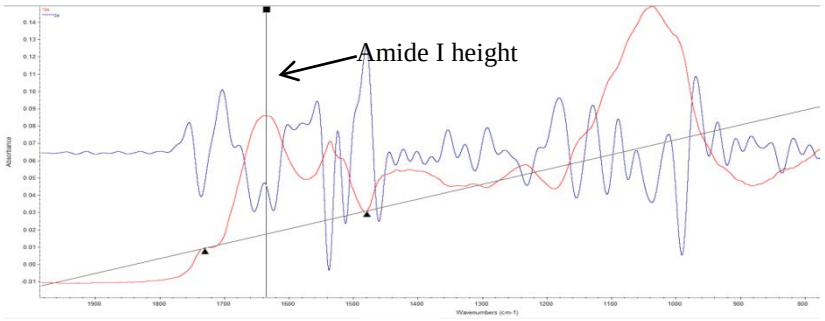
Table 8.3 Correlations between carbohydrate ATR-FTIR spectral profiles and predicted nutrient supply with DVE/OEB Dutch and NRC 2001 models in pelleting-processed canola meal

Items	Structural CHO peak 1 height	Structural CHO peak 2 height	Structural CHO peak 3 height	Structural CHO area	cellulosic height	cellulosic area	Total CHO peak 1 height	Total CHO peak 2 height	Total CHO peak 3 height	Total CHO peak 1 area	Total CHO peak 2 area	Total CHO peak 3 area	Total CHO area
						Spearman Correlation R values							
Estimation of nutrients supply using DVE/OEB													
FOM	0.00	-0.35	-0.54	-0.32	-0.69^{+}	-0.62	-0.13	-0.55	-0.53	-0.69^{+}	-0.55	-0.54	-0.56
TPSI	0.10	0.53	0.65	0.49	0.77*	0.71^{+}	0.15	0.59	0.68^{+}	0.83*	0.65	0.70^{+}	0.71^{+}
ENDP	-0.27	-0.55	-0.60	-0.60	-0.58	-0.61	0.07	-0.26	-0.68^{+}	-0.70^{+}	-0.59	-0.70^{+}	-0.69^{+}
N_MCP	0.00	-0.49	-0.60	-0.41	-0.74^{+}	-0.67^{+}	-0.04	-0.64	-0.63	-0.79*	-0.60	-0.66	-0.66
AMCP^{DVE}	-0.79*	-0.67	-0.74^{+}	-0.74^{+}	-0.70^{+}	-0.71^{+}	-0.35	0.55	-0.69^{+}	-0.65	-0.65	-0.63	-0.64
BCP	0.09	0.51	0.64	0.47	0.77*	0.70^{+}	0.15	0.59	0.67^{+}	0.82*	0.64	0.69^{+}	0.70^{+}
ABCP^{DVE}	0.22	0.59	0.72^{+}	0.60	0.81*	0.77*	0.20	0.50	0.77*	0.90**	0.71^{+}	0.78*	0.78*
DVE	0.23	0.61	0.73^{+}	0.62	0.81*	0.77*	0.20	0.49	0.78*	0.91**	0.72^{+}	0.79*	0.79*
OEB^{DVE}	0.00	-0.50	-0.60	-0.42	-0.74^{+}	-0.67	-0.03	-0.65	-0.64	-0.79*	-0.60	-0.66	-0.67
Estimation of nutrients supply using the NRC 2001													
MCP$_{TDN}$	0.74^{+}	0.95*	0.73^{+}	0.81*	0.67^{+}	0.62	0.30	-0.35	0.77*	0.70^{+}	0.63	0.73^{+}	0.72^{+}
MCP^{NRC}	0.74^{+}	0.95*	0.73^{+}	0.81*	0.67^{+}	0.62	0.30	-0.35	0.77*	0.70^{+}	0.63	0.73^{+}	0.72^{+}
AMCP^{NRC}	0.73^{+}	0.77*	0.83*	0.73^{+}	0.81*	0.80*	0.11	-0.42	0.77*	0.67^{+}	0.79*	0.74^{+}	0.75^{+}
RUP^{NRC}	0.10	0.52	0.65	0.48	0.77*	0.71^{+}	0.16	0.59	0.68^{+}	0.82*	0.65	0.70^{+}	0.71^{+}
ARUP^{NRC}	0.22	0.59	0.72^{+}	0.60	0.81*	0.77*	0.12	0.50	0.77*	0.90**	0.71^{+}	0.78*	0.78*
ECP	0.06	0.66	0.62	0.45	0.75^{+}	0.68^{+}	-0.19	0.46	0.67	0.78*	0.60	0.69^{+}	0.69^{+}
AECP^{NRC}	0.05	0.65	0.62	0.44	0.74^{+}	0.67^{+}	-0.19	0.48	0.66	0.78*	0.59	0.68^{+}	0.68^{+}
MP^{NRC}	0.22	0.60	0.73^{+}	0.61	0.82*	0.77*	0.19	0.49	0.77*	0.90**	0.71^{+}	0.78*	0.78*
OEB^{NRC}	-0.01	-0.50	-0.60	-0.41^{+}	-0.74^{+}	-0.68^{+}	-0.03	-0.64	-0.64	-0.79*	-0.61	-0.66	-0.67

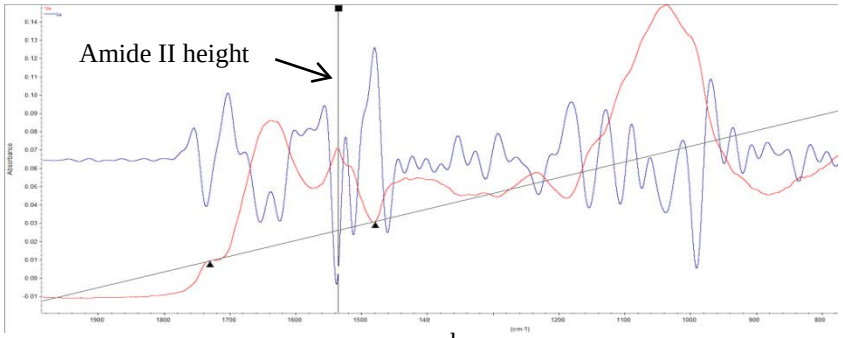
Notes: n=14. R: Correlation coefficient calculated using spearman method; FOM: fermented organic matter in the rumen; TPSI: total protein supplied to the small intestine; ENDP: endogenous protein in the small intestine; N_MCP: microbial protein synthesized based on available nitrogen; AMCP^{DVE}: truly absorbed microbial protein in the small intestine; BCP: rumen bypass protein; ABCP^{DVE}: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance in DVE/OEB model; MCP$_{TDN}$: microbial protein synthesized in the rumen based on discounted TDN; MCP^{NRC}: microbial protein; AMCP^{NRC}: truly absorbed microbial protein in the small intestine; RUP^{NRC}: rumen undegradable feed crude protein; ARUP^{NRC}: truly absorbed rumen undegradable protein in the small

Table 8.3 Cont'd

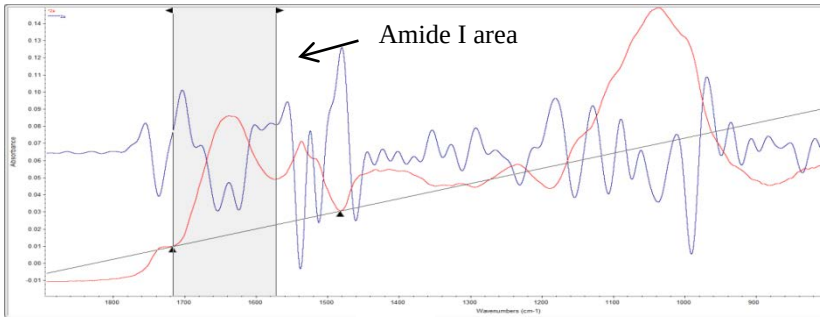
intestine; ECP: rumen endogenous protein; $AECP^{NRC}$: truly absorbed rumen endogenous protein in the small intestine; MP^{NRC}: metabolizable protein. OEB^{NRC}: degraded protein balance in the NRC 2001 model. "+": P<0.10; "*": P<0.05 "**": P<0.01.


Amide I height

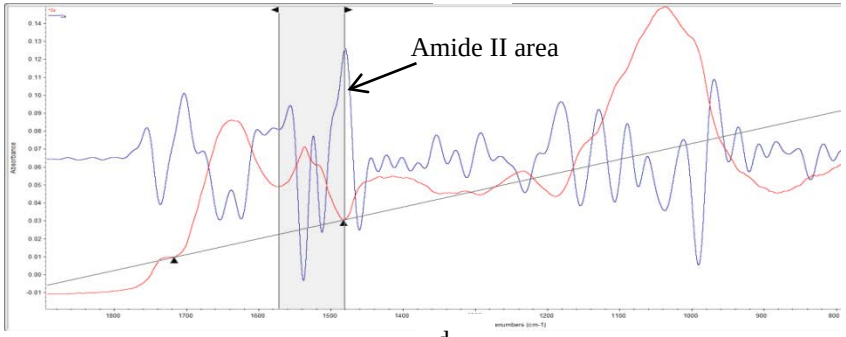
a.


Amide II height

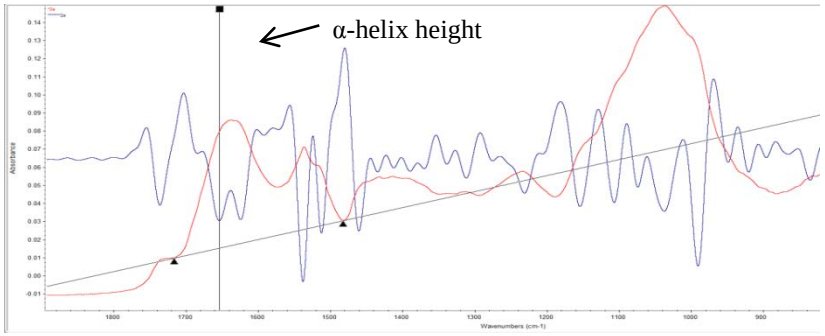
b.


Amide I area

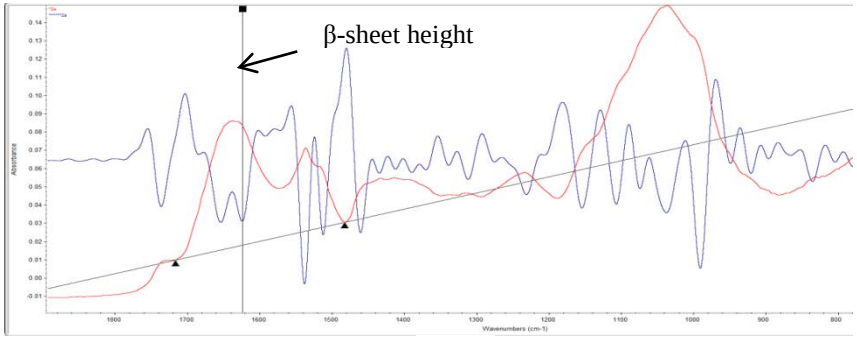
c.


Amide II area

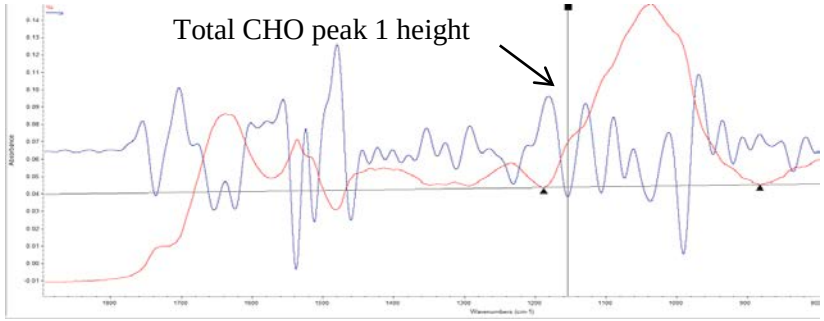
d.


α-helix height

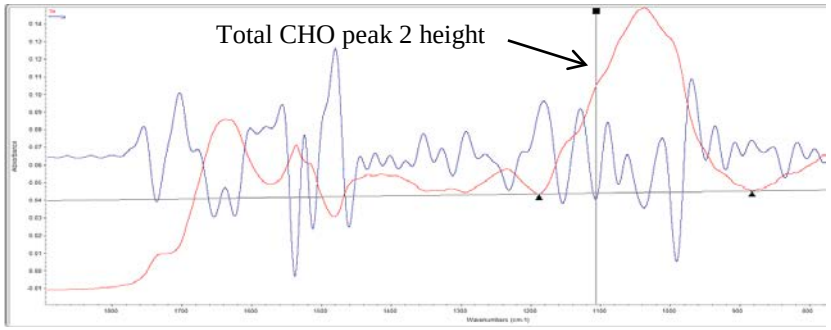
e.


β-sheet height

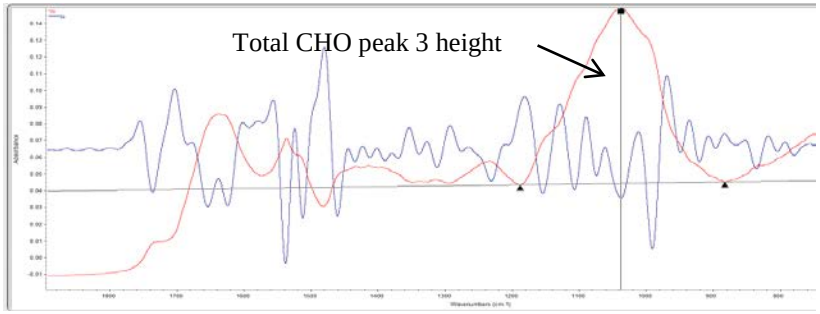
f.


Total CHO peak 1 height

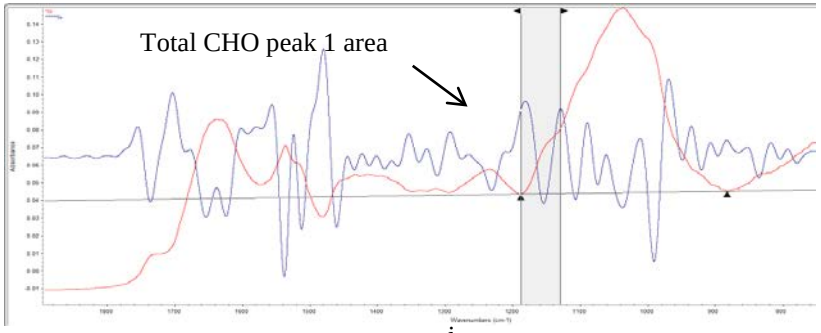
g.


Total CHO peak 2 height

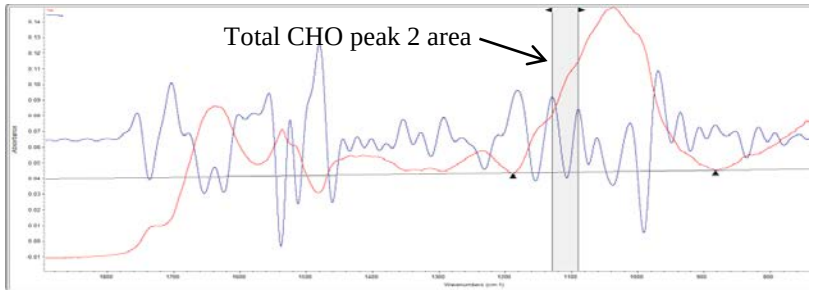
h.


Total CHO peak 3 height

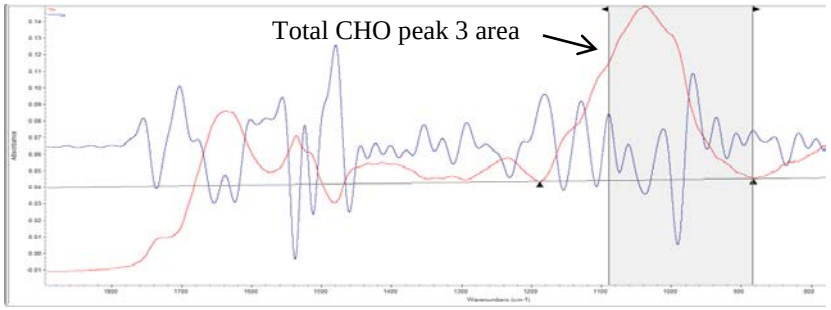
i.


Total CHO peak 1 area

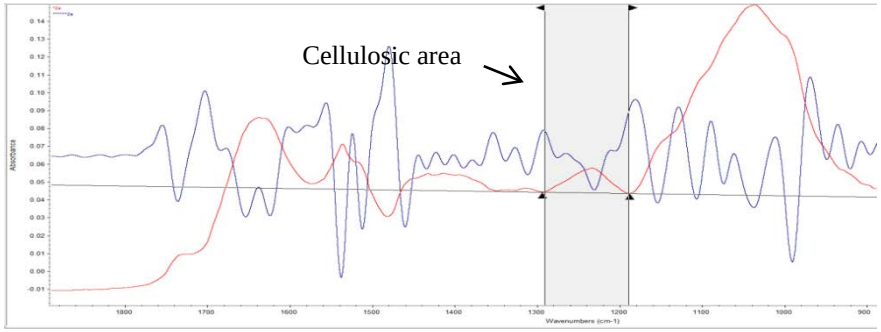
j.


Total CHO peak 2 area

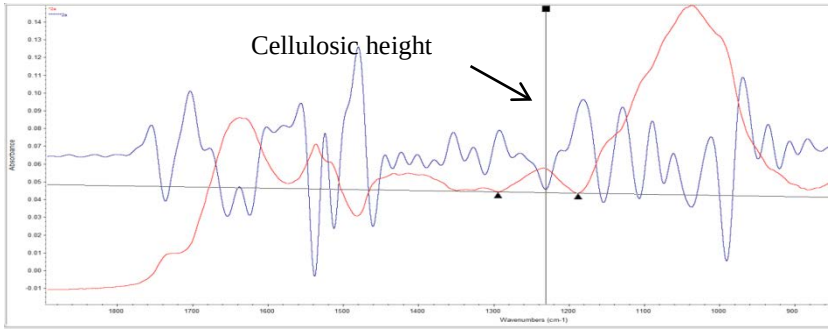
k.


Total CHO peak 3 area

l.

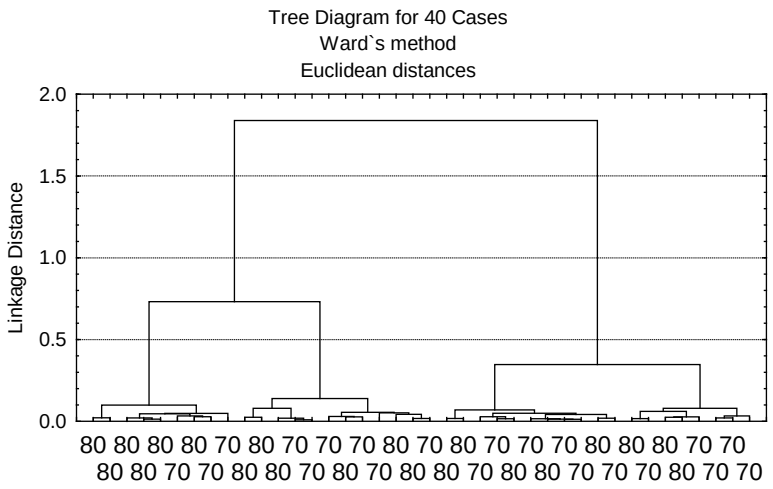


m.

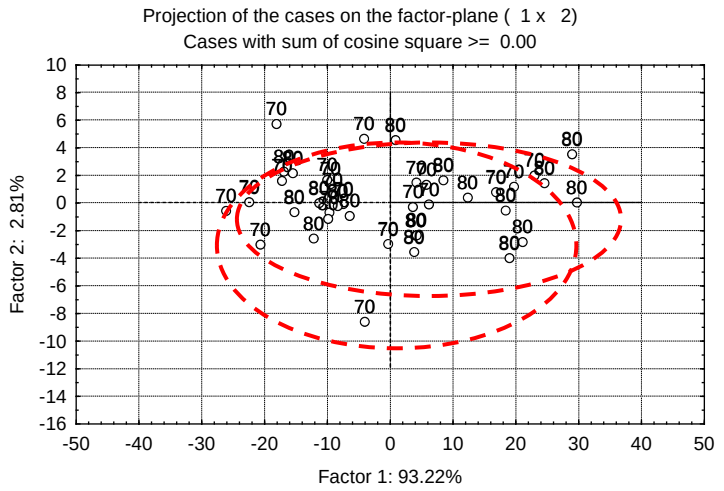


n.

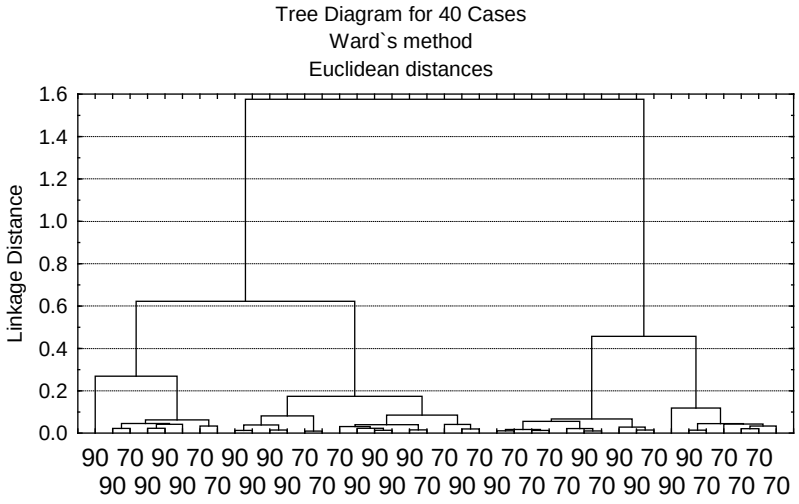
Figure 8.1 Typical FTIR spectrum for canola meal at the regions of protein (a-f; ca. 1718-1481 cm^{-1}), total carbohydrates (TCHO) (g-l; 1193-879 cm^{-1}), cellulosic compounds (CEL) (m-n; ca. 1302-1186 cm^{-1})



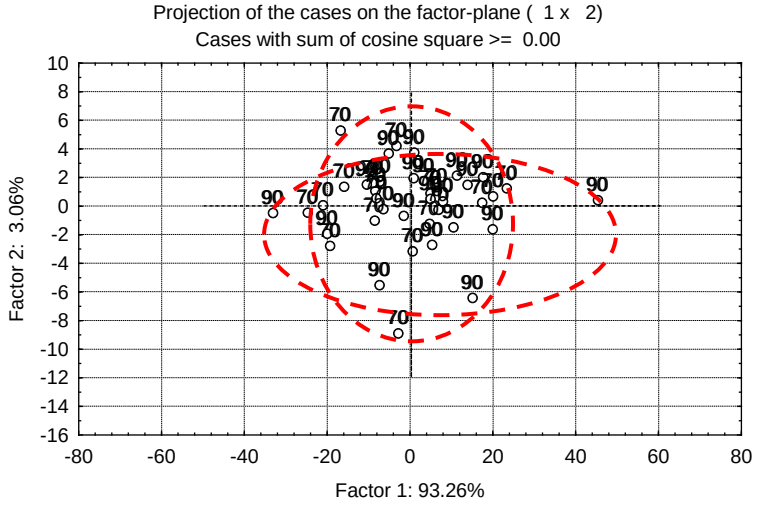
(a). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



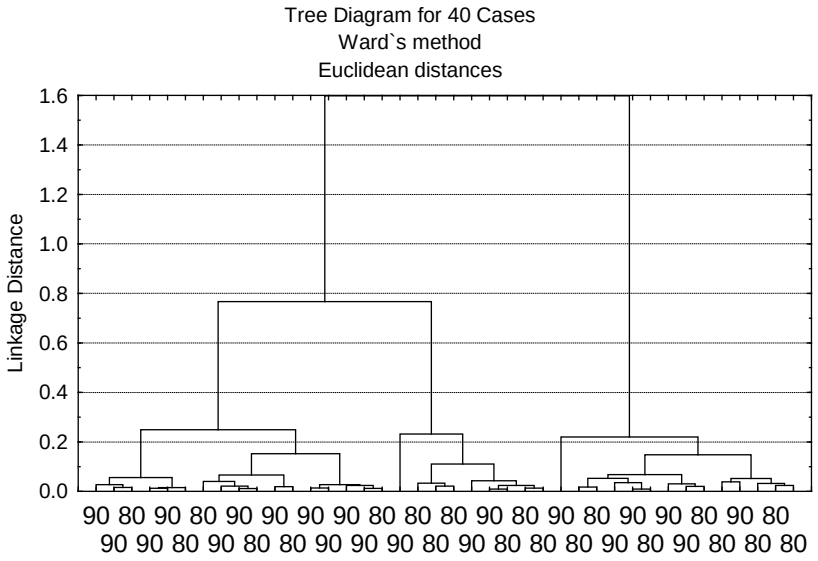
(b). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80 n=20)



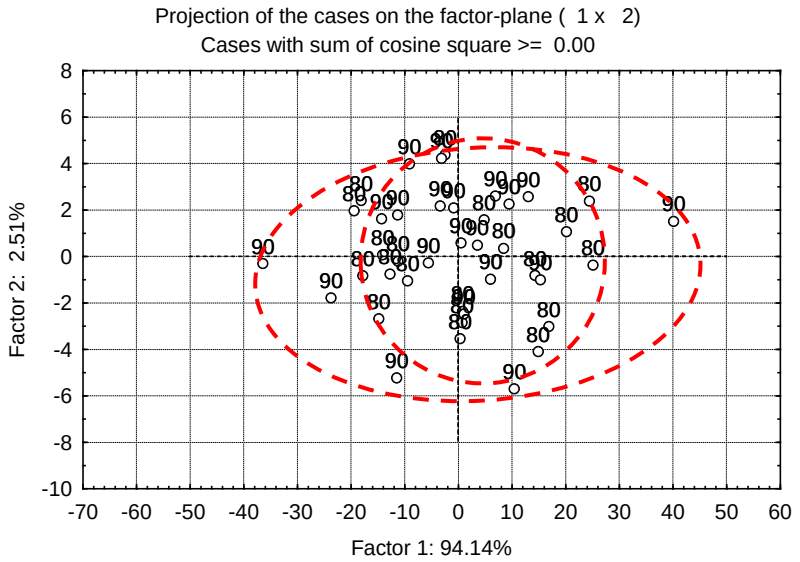
(c). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



(d). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90 n=20)

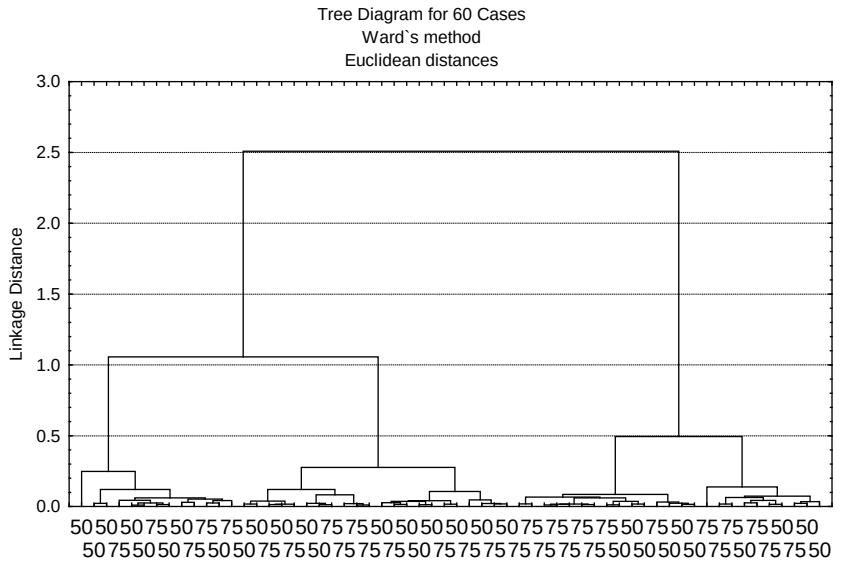


(e). CLA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)

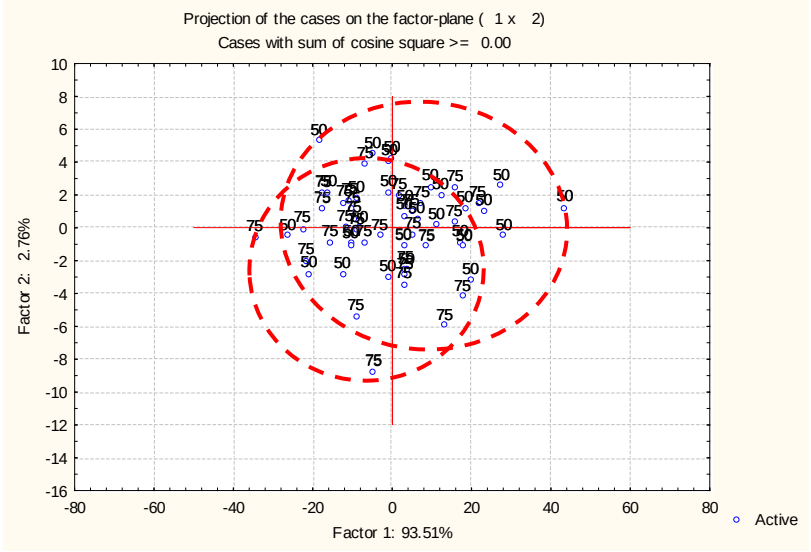


(f). PCA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)

Figure 8.2 Multivariate molecular spectral analyses of protein fingerprint region at ca. 1718-1481 cm^{-1}: comparison of samples processed under different temperatures. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).

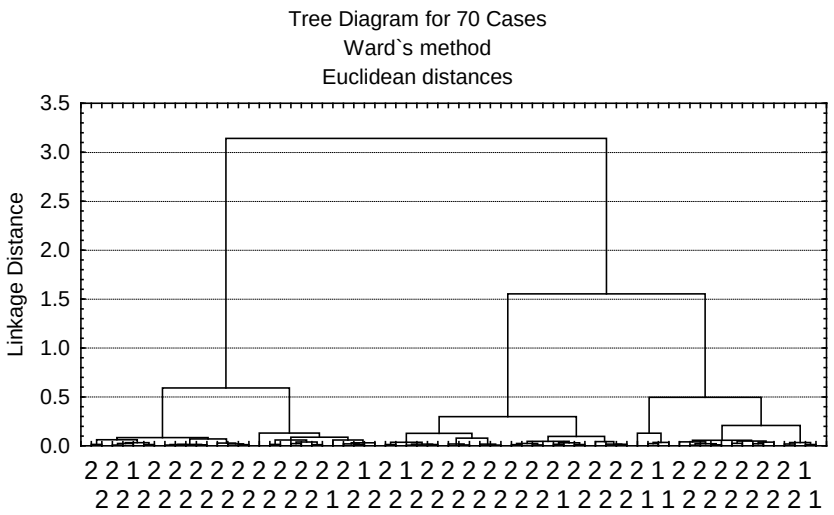


(a). CLA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec (75; n=30)

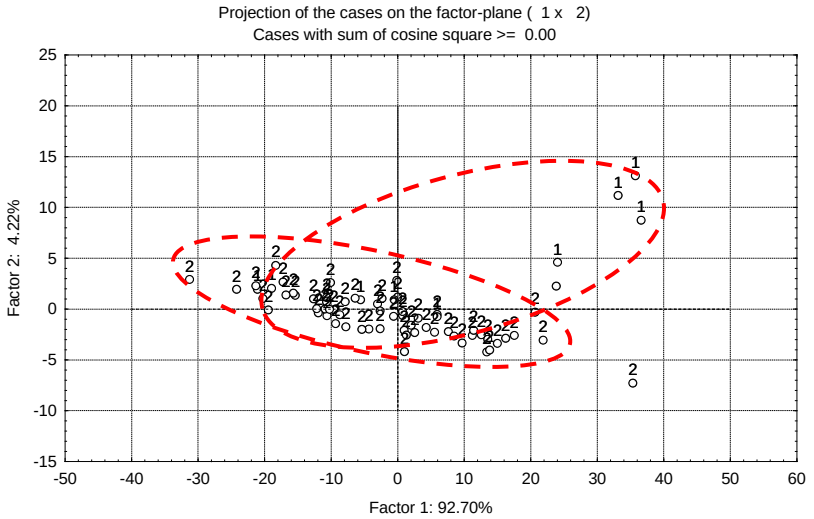


(b). PCA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec(75; n=30)

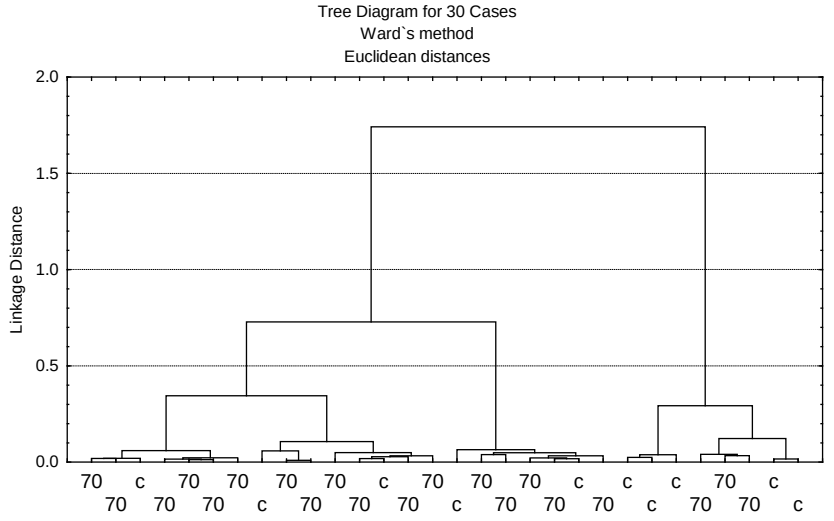
Figure 8.3 Multivariate molecular spectral analyses of protein fingerprint region at ca. 1718-1481 cm^{-1}: comparison of samples processed under different time. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).



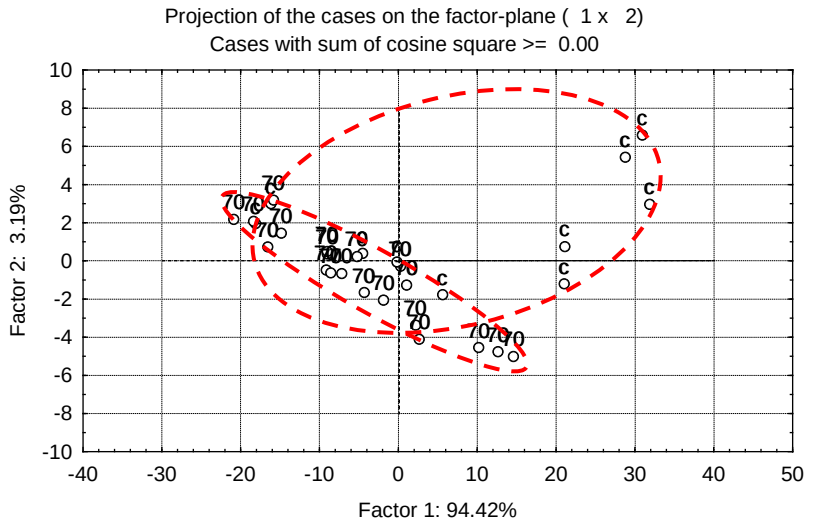
(a). CLA: unprocessed mash (1; n=10) vs. pellets (2; n=60)



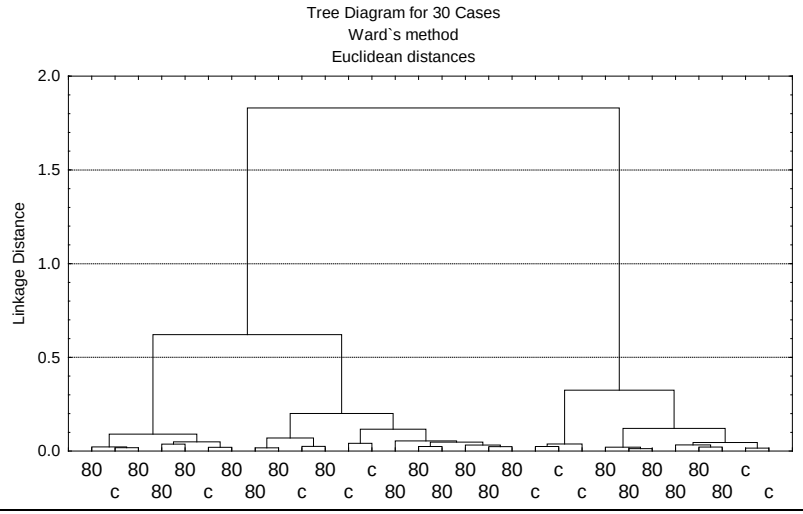
(b). PCA: unprocessed mash (1; n=10) vs. pellets (2; n=60)



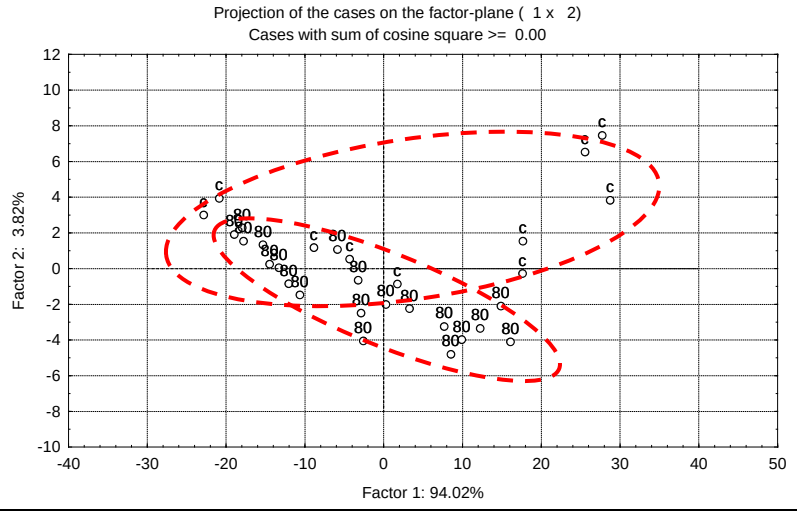
(c). CLA: unprocessed mash (c; n=10) vs. samples conditioned at 70ºC (70; n=20)



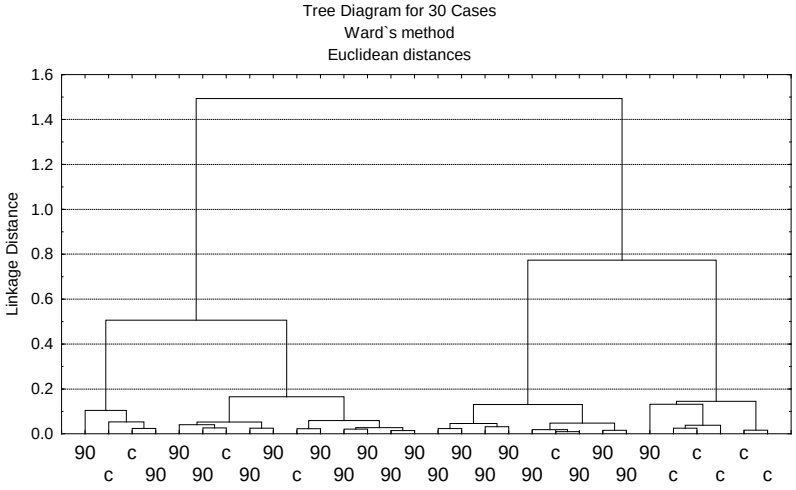
(d). PCA: unprocessed mash (c) vs. samples conditioned at 70ºC (70; n=20)



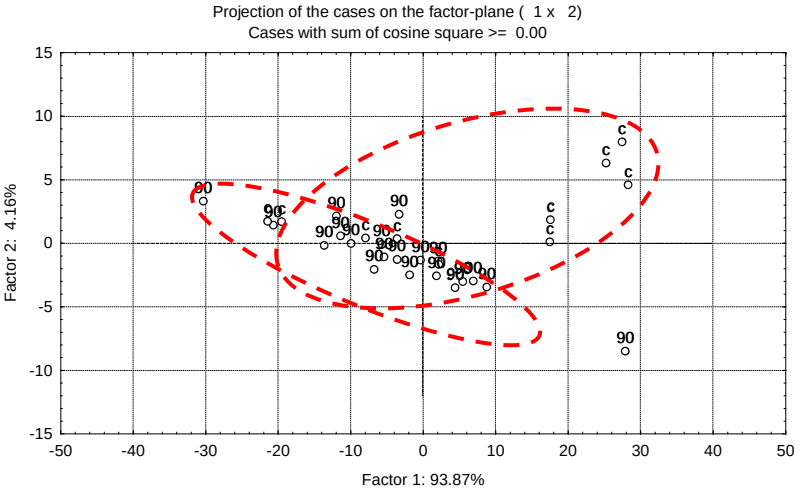
(e). CLA: unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80;n=20)



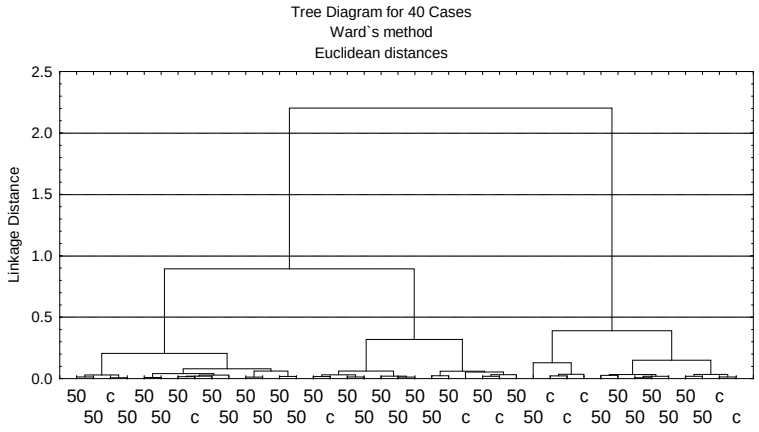
(f). PCA: unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



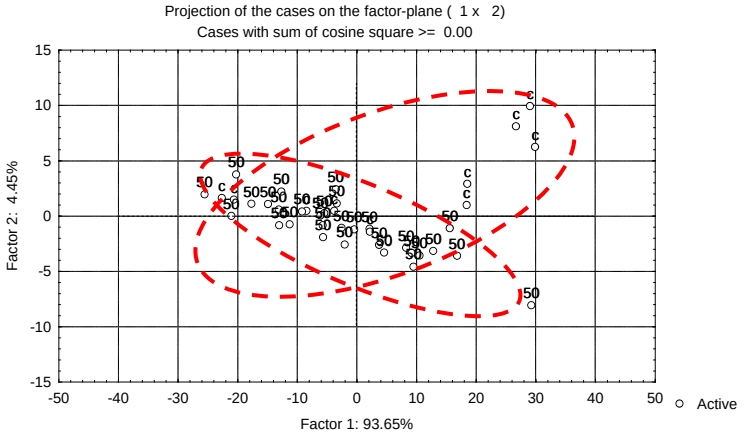
(g). CLA: unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



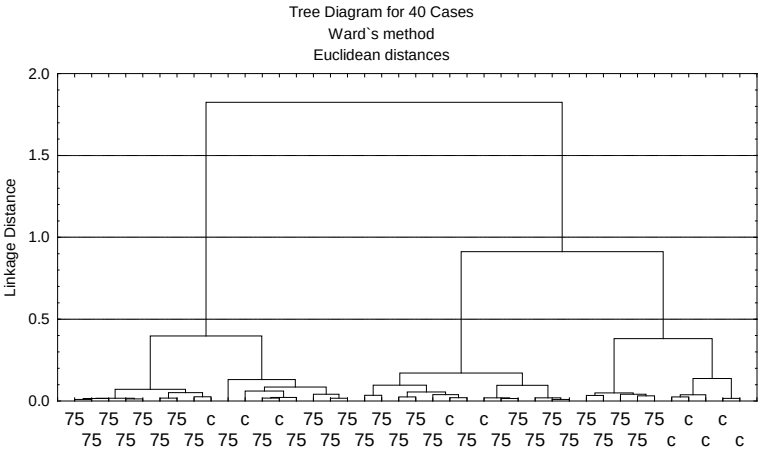
(h). PCA: unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



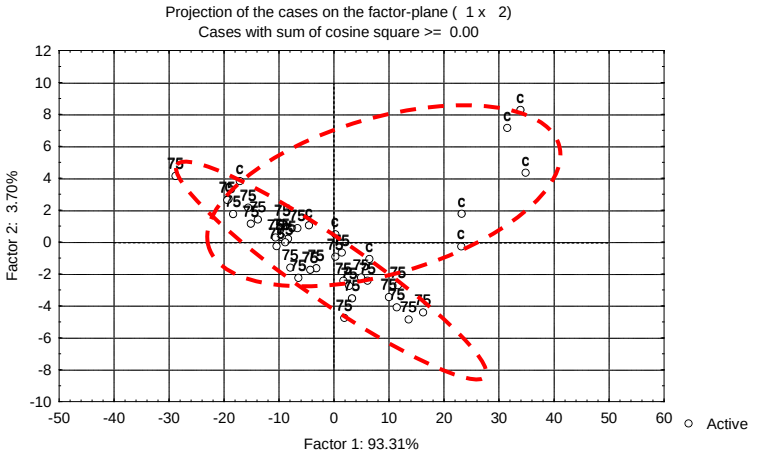
(i). CLA: unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)



(j). PCA: unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)

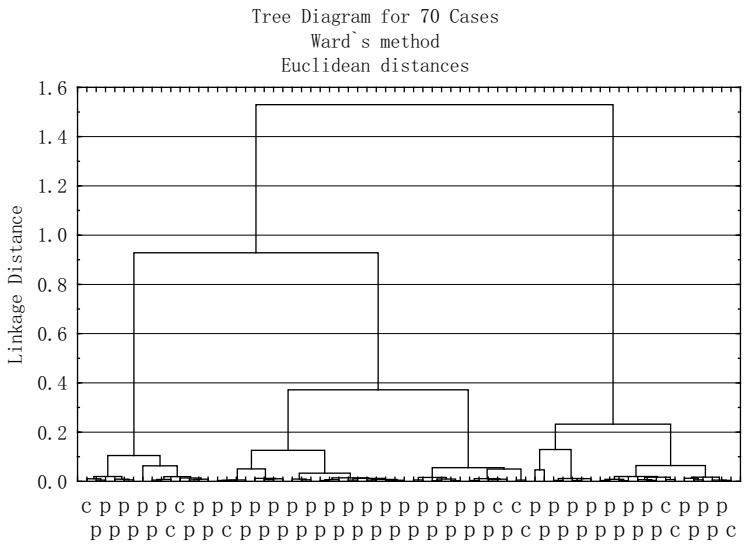


(k). CLA: unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)

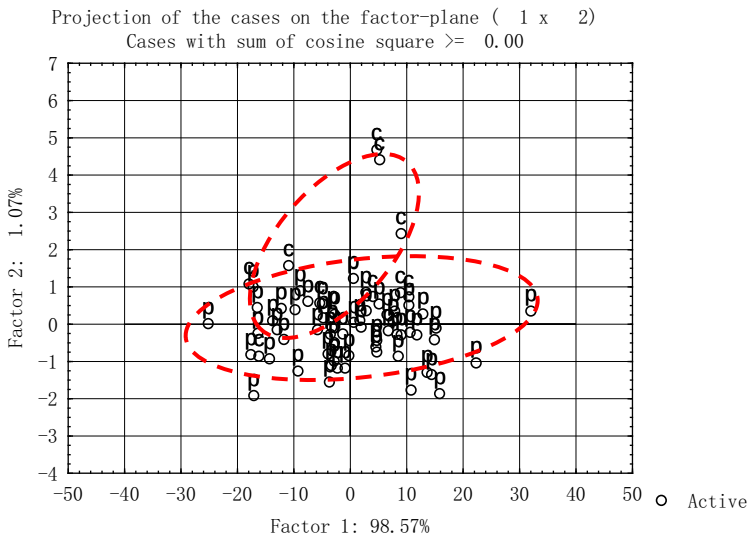


(l). PCA: unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)

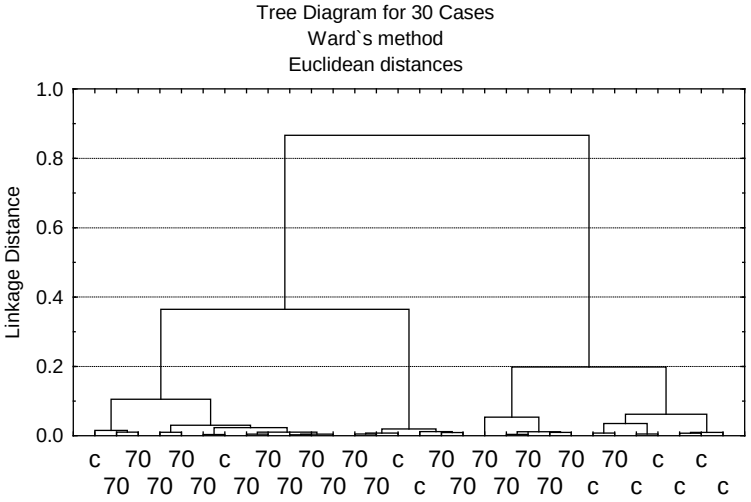
Figure 8.4 Multivariate molecular spectral analyses of protein fingerprint region at ca. 1718-1481 cm^{-1}: comparison of the unprocessed mash to samples conditioned under different conditions. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).



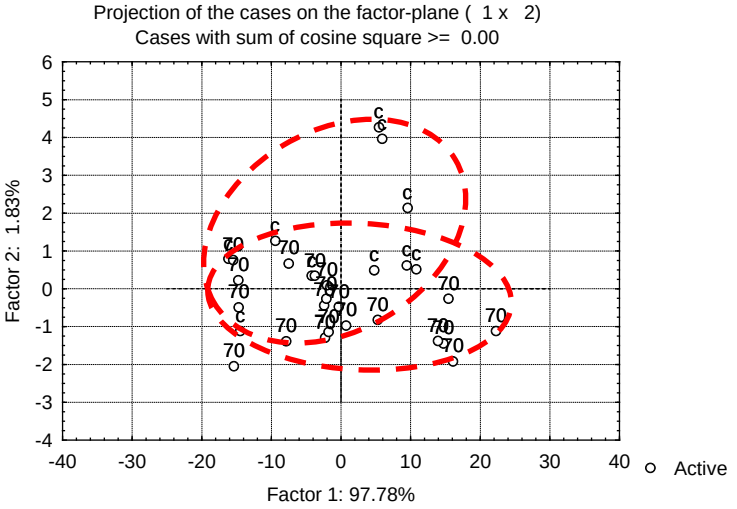
(a). CLA: the unprocessed mash (c; n=10) vs. pellets (p; n=60)



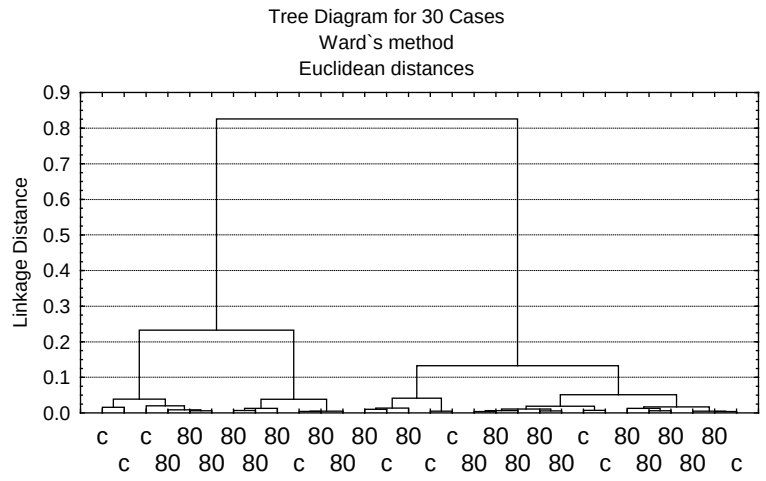
(b). PCA: the unprocessed mash (c; n=10) vs. pellets (p; n=60)



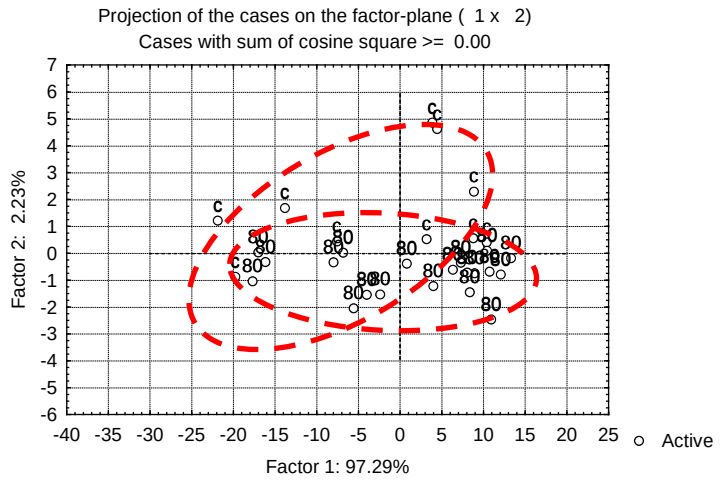
(c). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 70ºC (70; n=20)



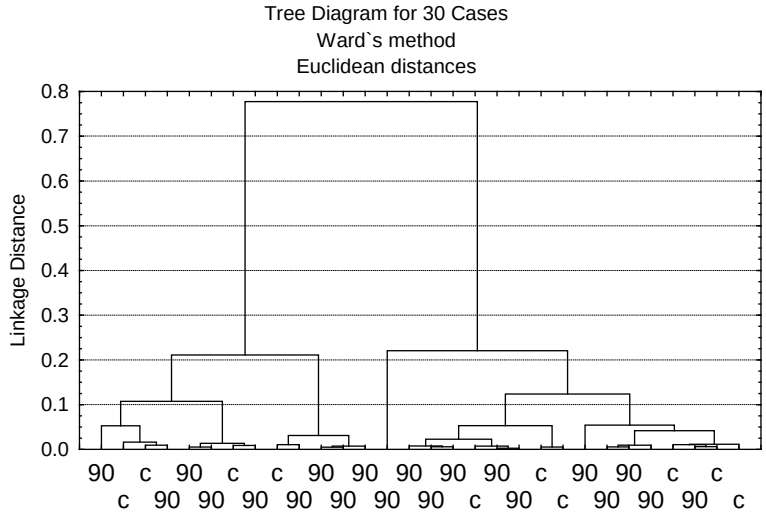
(d). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 70ºC (70; n=20)



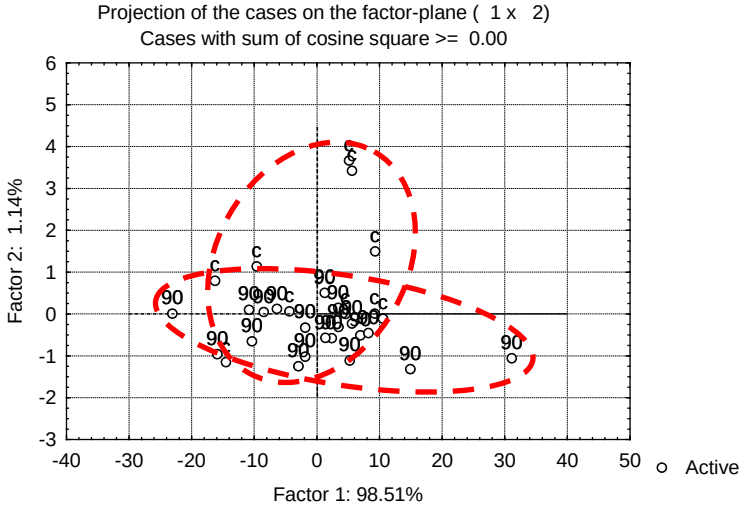
(e). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



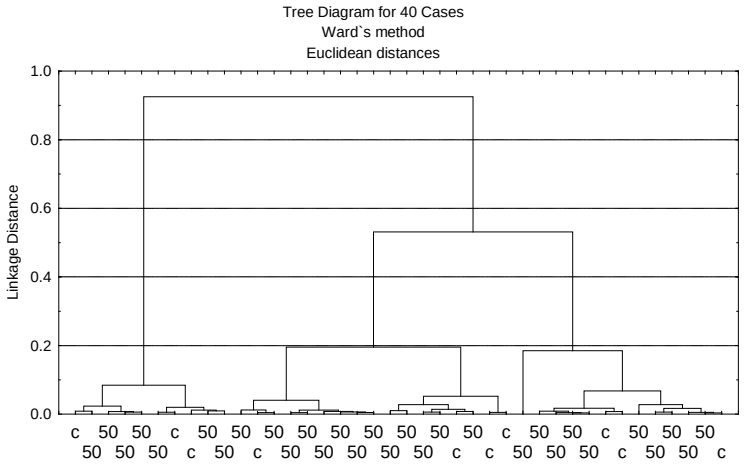
(f). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



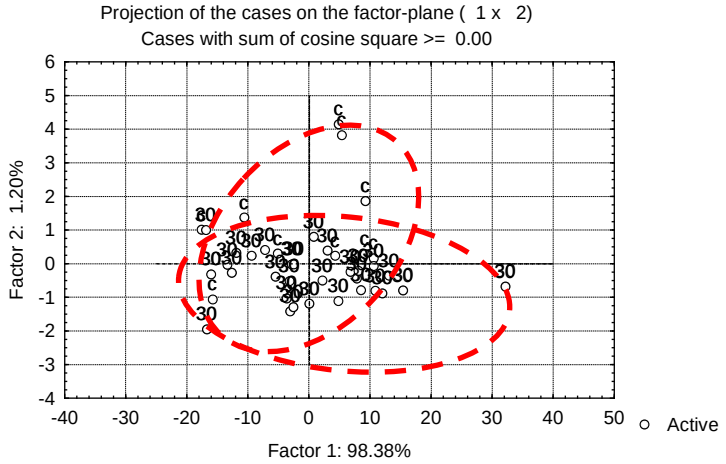
(g). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



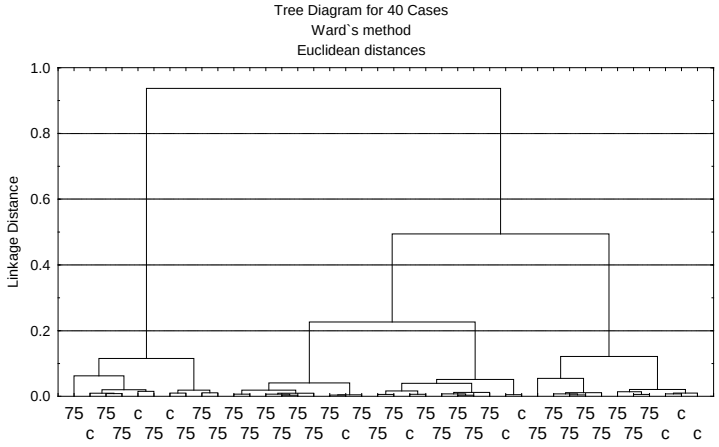
(h). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



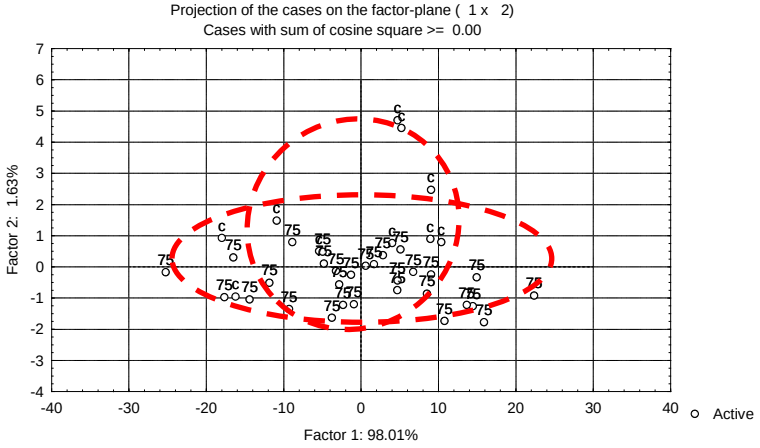
(i). CLA: the unprocessed mash (c; c=10) vs. samples conditioned for 50 sec (50; n=30)



(j). PCA: the unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)

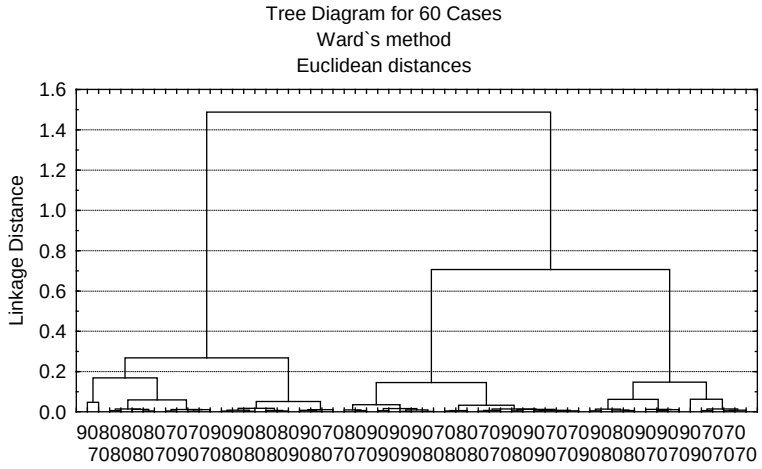


(k). CLA: the unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)

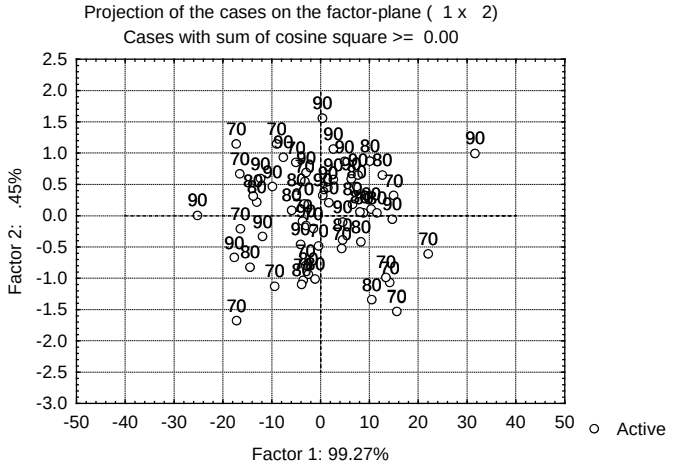


(l). PCA: the unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)

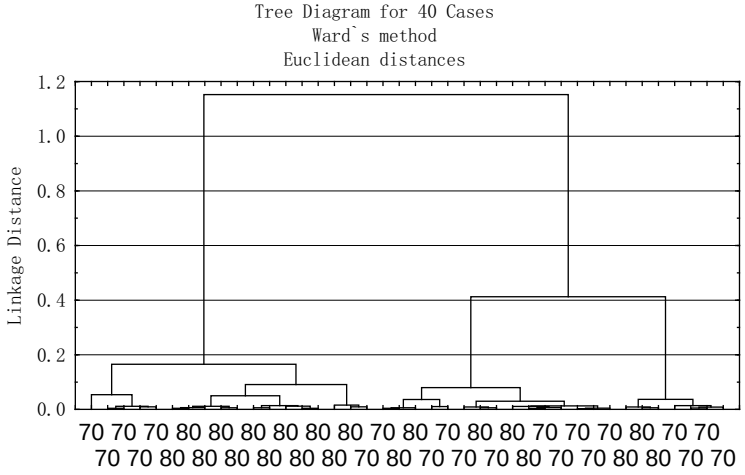
Figure 8.5 Multivariate molecular spectral analyses of cellulosic compounds region at ca. 1302-1186 cm^{-1}: comparison of the unprocessed mash to samples conditioned under different conditions. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).



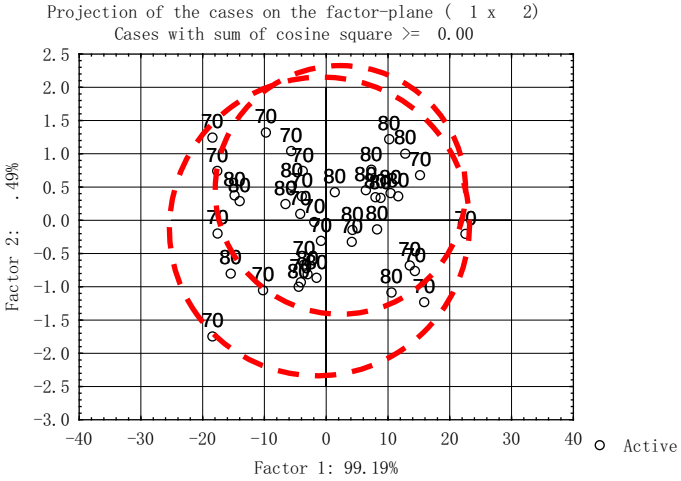
(a). CLA:70ºC (70; n=20), 80ºC (80; n=20), and 90ºC (90; n=20)



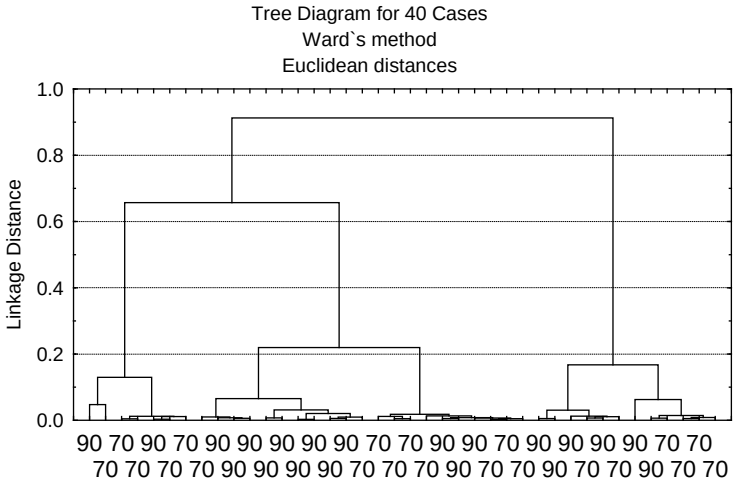
(b).PCA: 70ºC (70; n=20), 80ºC (80; n=20), and 90ºC (90; n=20)



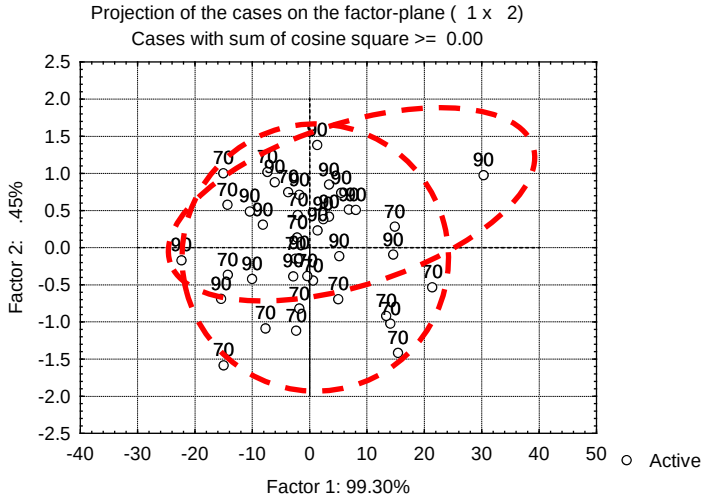
(c). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



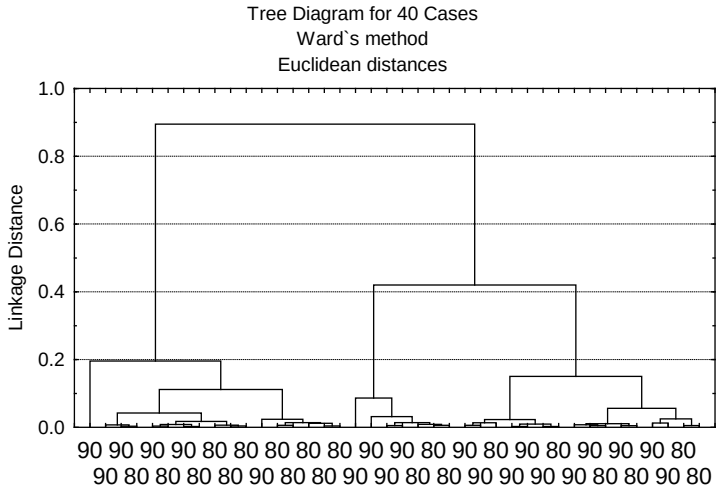
(d). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



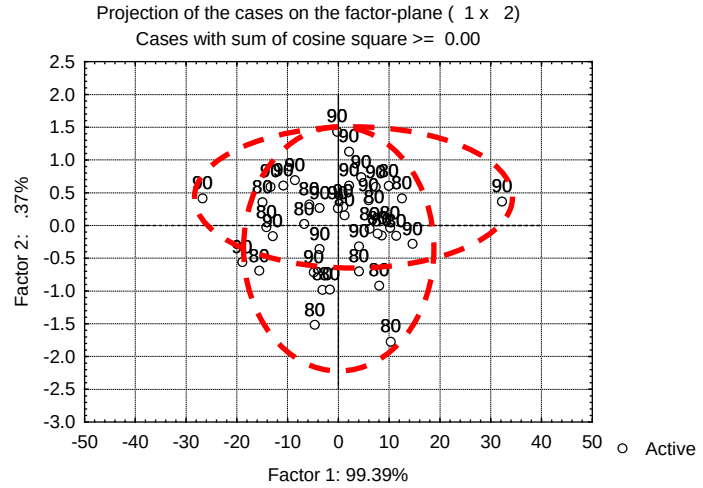
(e). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



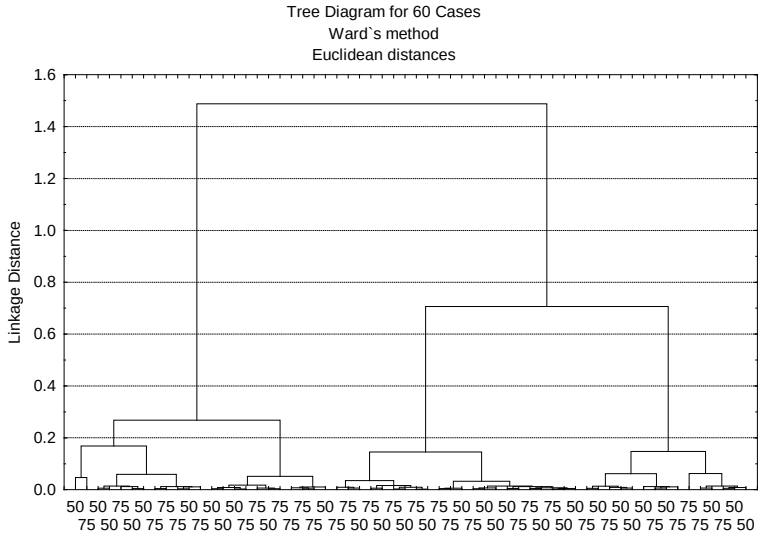
(f). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



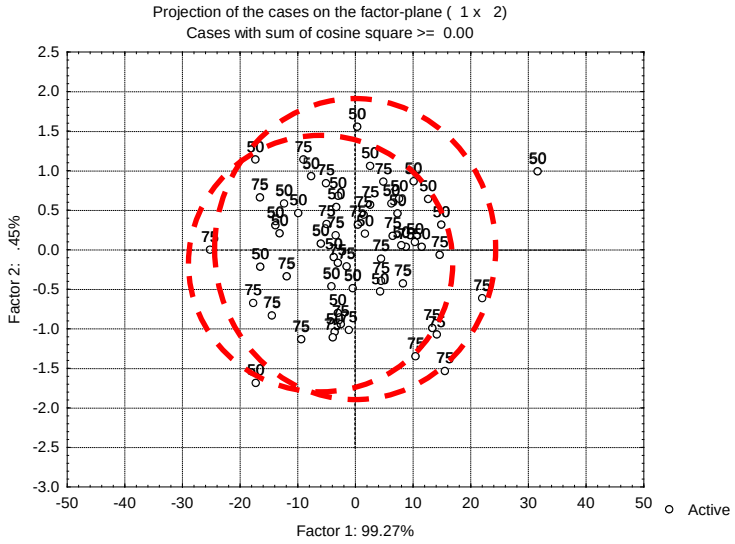
(g). CLA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)



(h). PCA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)

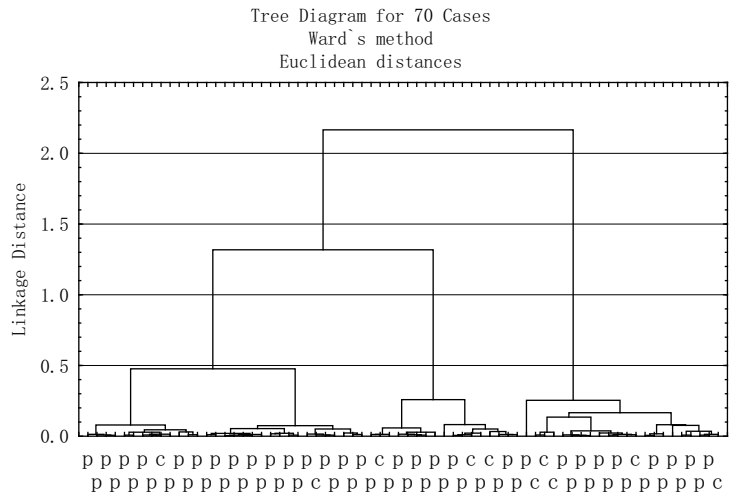


(i). CLA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec (75; n=30)

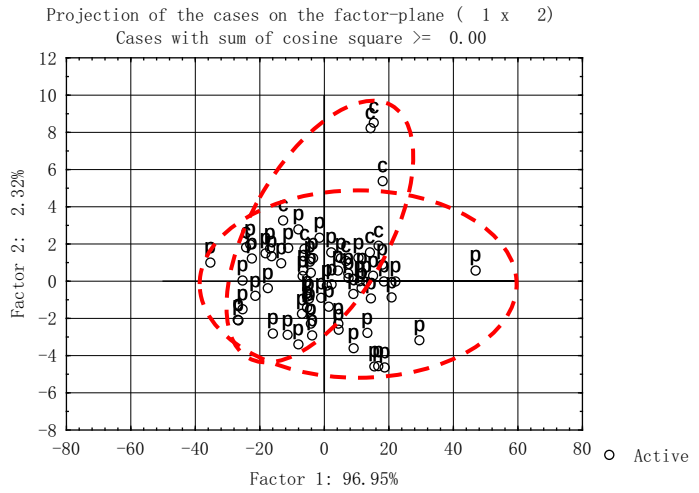


(j) PCA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned at 75 sec (75; n=30)

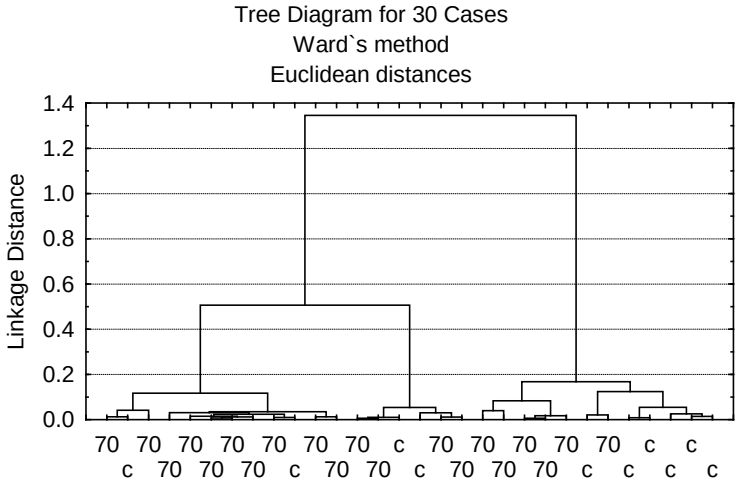
Figure 8.6 Multivariate molecular spectral analyses of cellulosic compounds region at ca. 1302-1186 cm^{-1}: comparison of samples processed under different conditions. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).



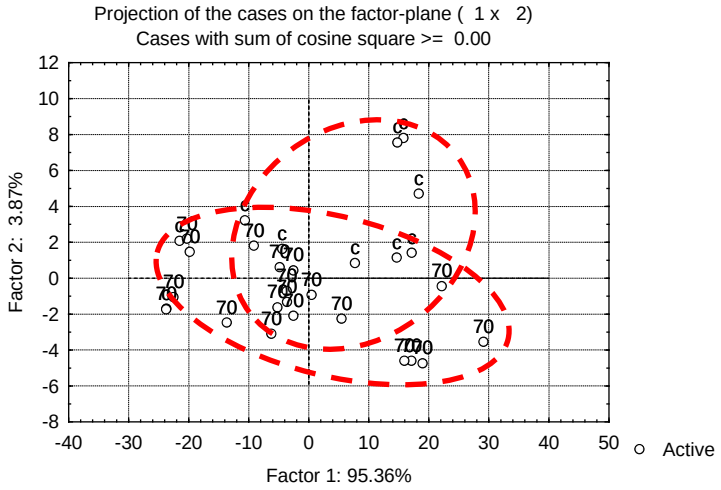
(a). CLA: the unprocessed sample (c; n=10) vs. pellets (p; n=60)



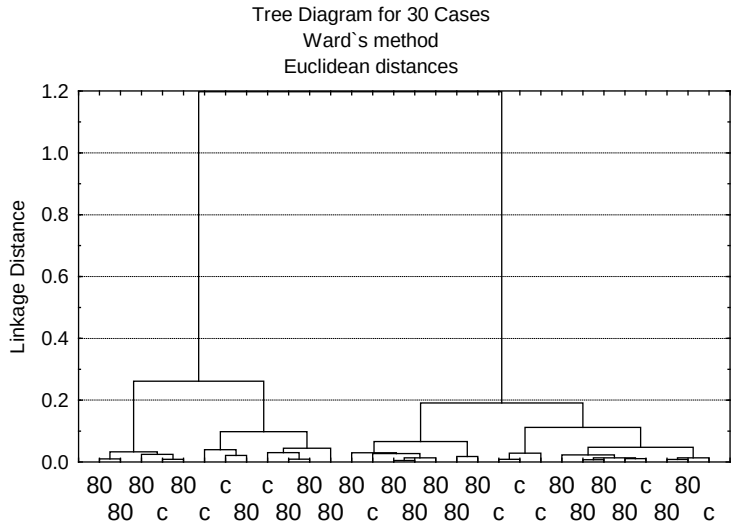
(b). PCA: the unprocessed sample (c; n=10) vs. pellets (p; n=60)



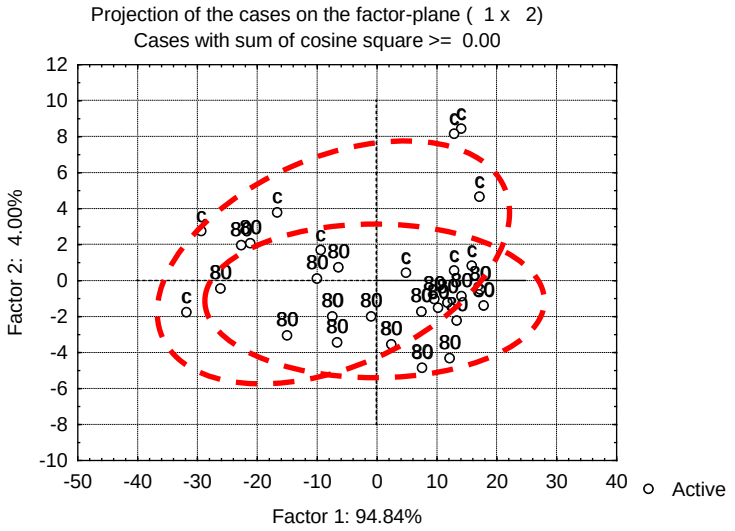
(c). CLA : the unprocessed sample (c; n=10) vs. samples conditioned at 70ºC (70; n=20)



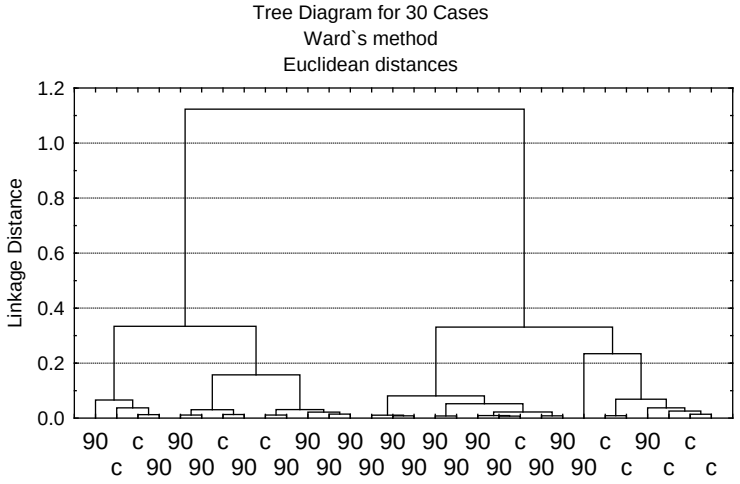
(d). PCA: the unprocessed sample (c) vs. samples conditioned at 70ºC (70; n=20)



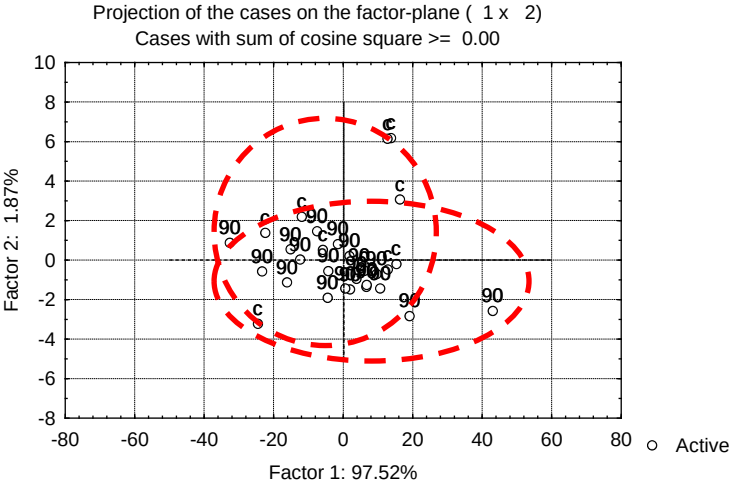
(e). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



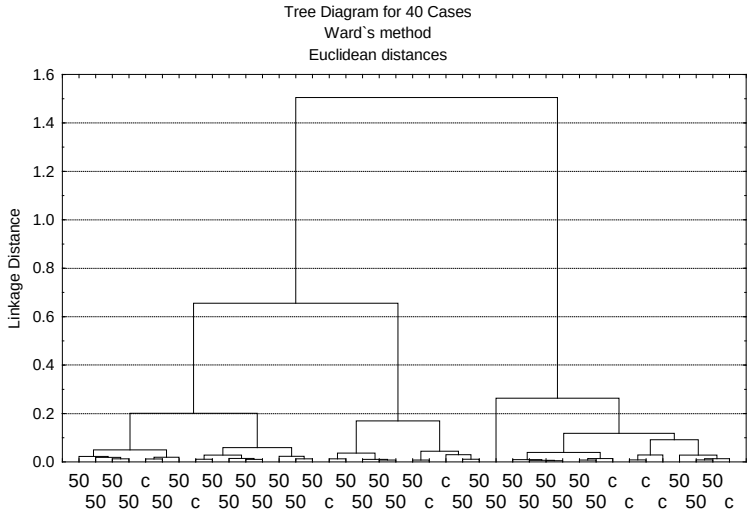
(f). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



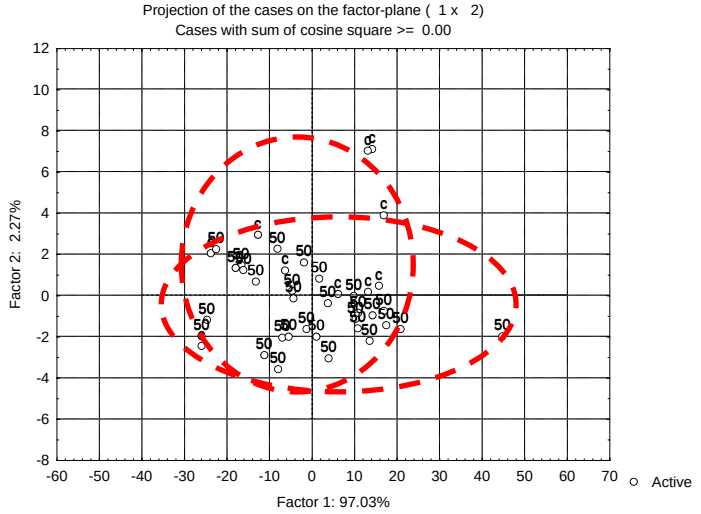
(g). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



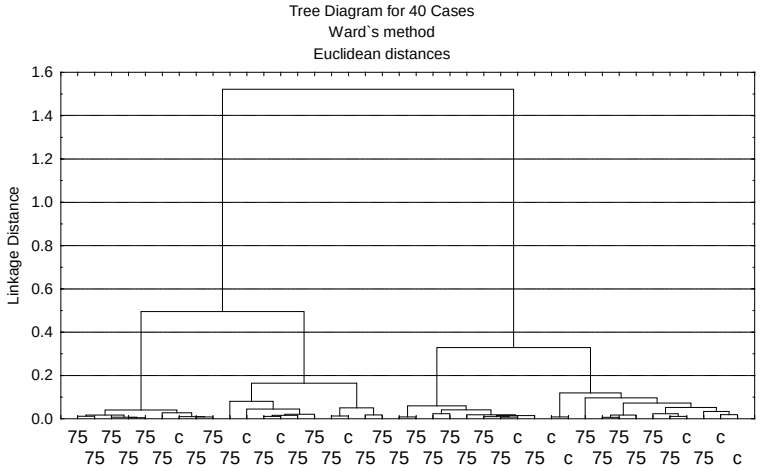
(h). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



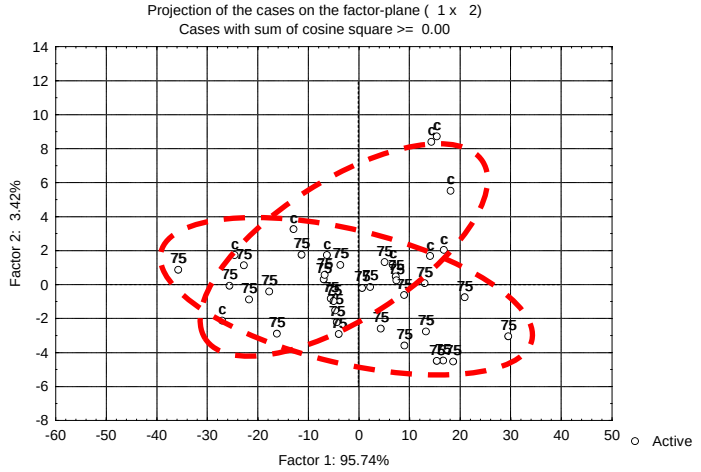
(i). CLA: the unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)



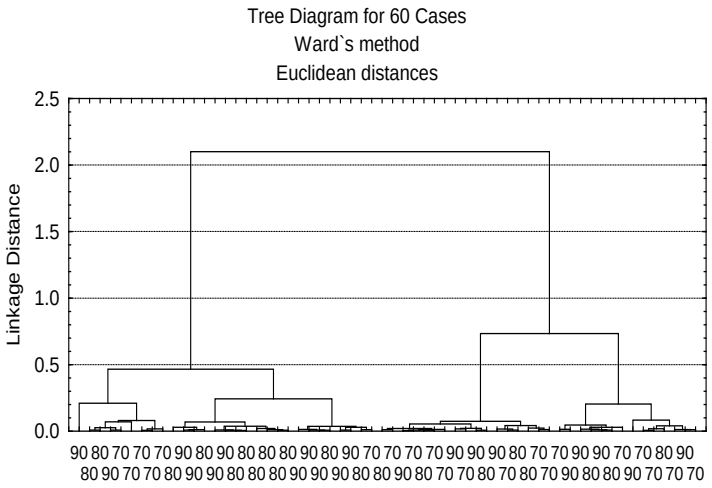
(j). PCA: the unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)



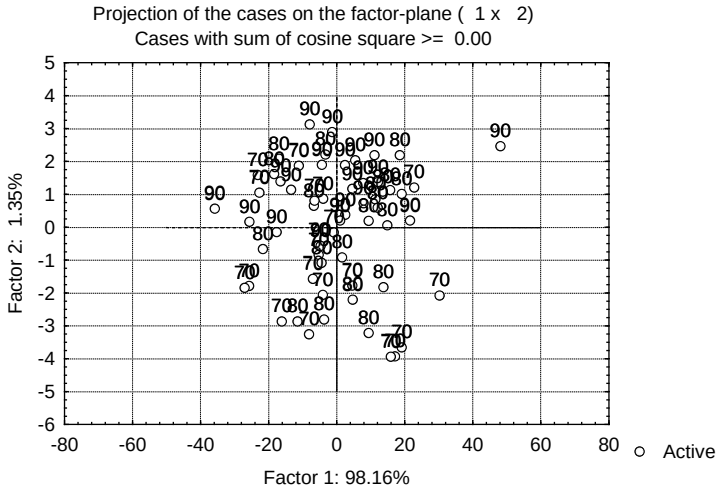
(k). CLA: the unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)



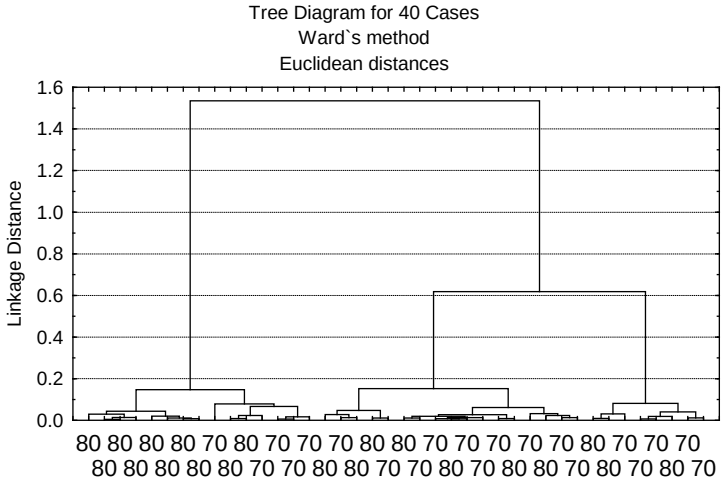
(l). PCA: the unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)



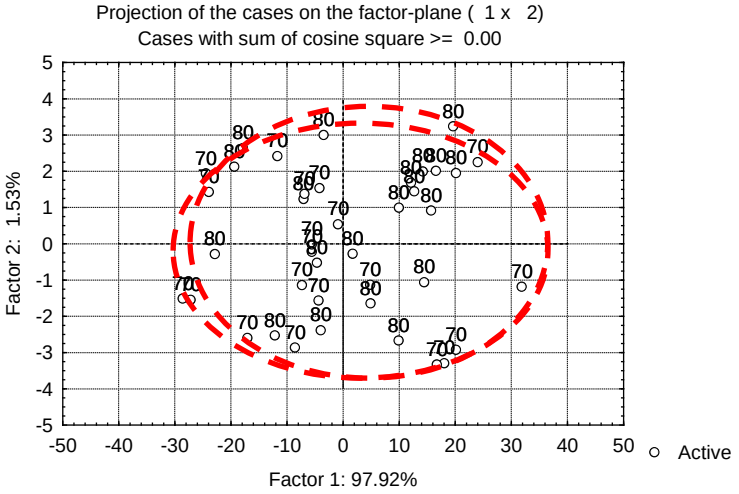
(m). CLA: samples conditioned at 70ºC (70; n=20), 80ºC (80; n=20) and 90ºC (90; n=20)



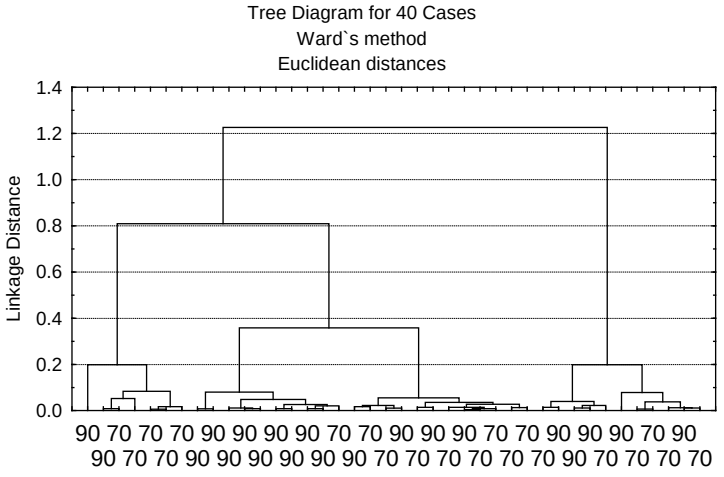
(n). PAC: samples conditioned at 70ºC (70; n=20), 80ºC (80; n=20) and 90ºC (90; n=20)



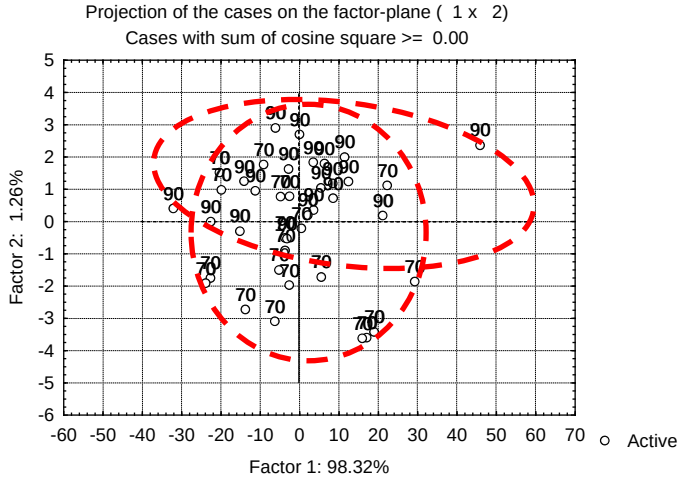
(o). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



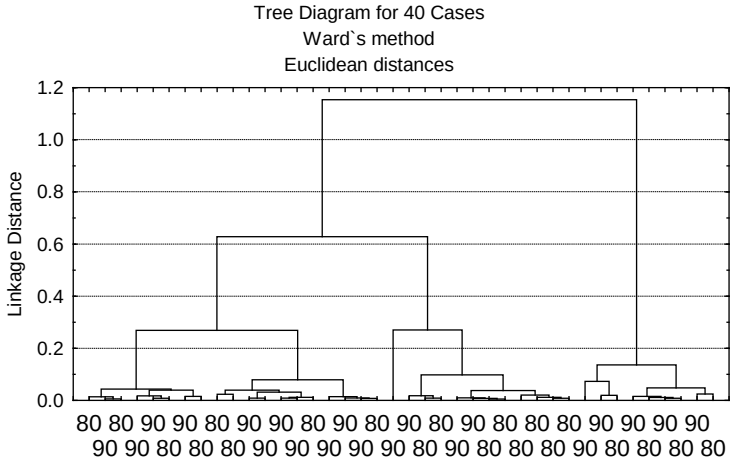
(p). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



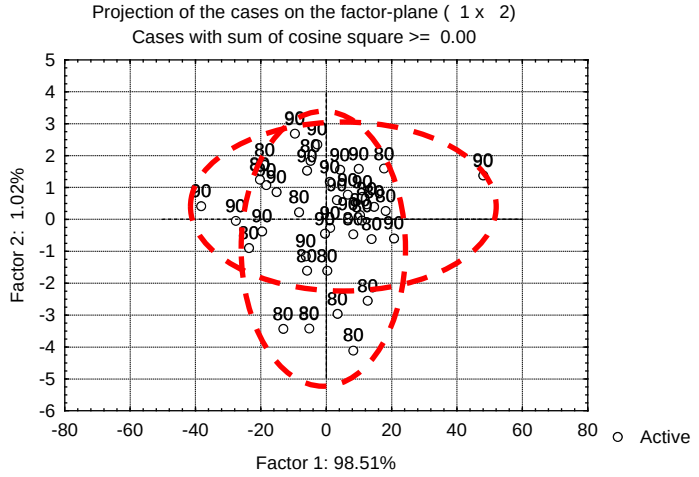
(q). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



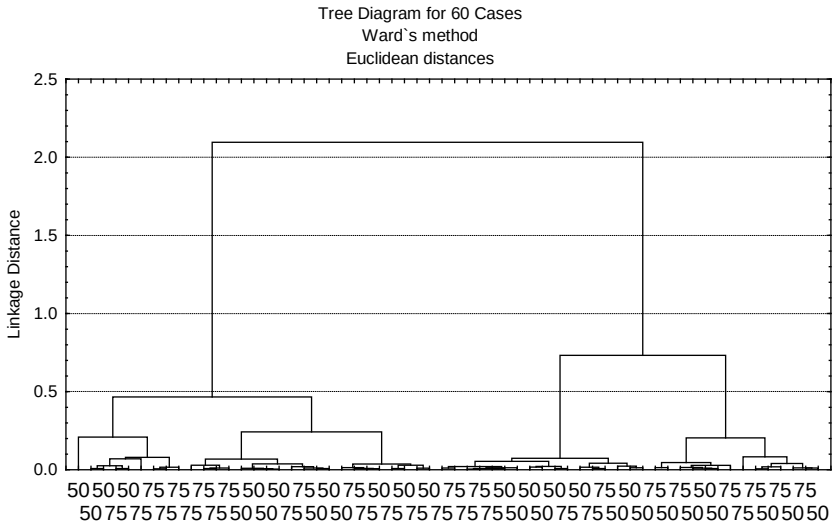
(r). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



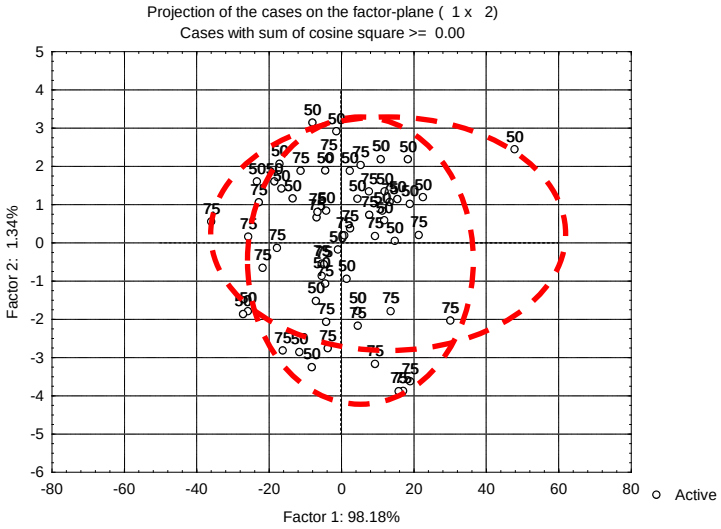
(s). CLA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)



(t). PCA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)

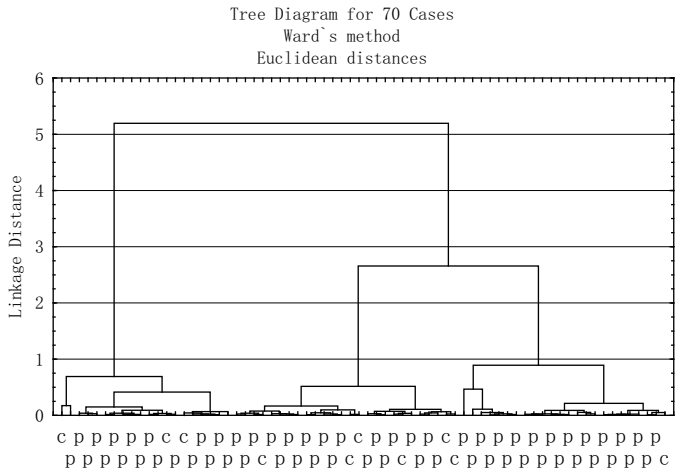


(u). CLA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec (75; n=30)

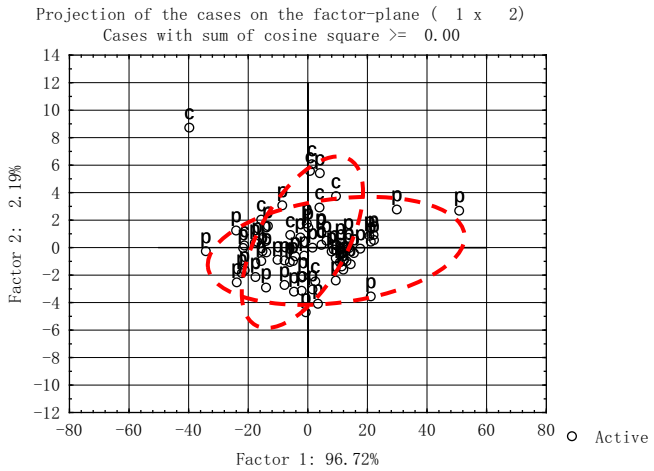


(v). PCA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec (75; n=30)

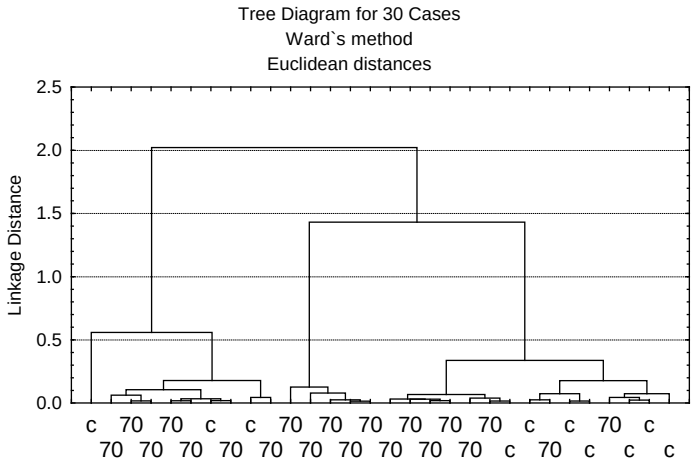
Figure 8.7 Multivariate molecular spectral analyses of SCHO fingerprint region at: 1488-1186 cm^{-1}: comparison of samples conditioned at different conditions. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).



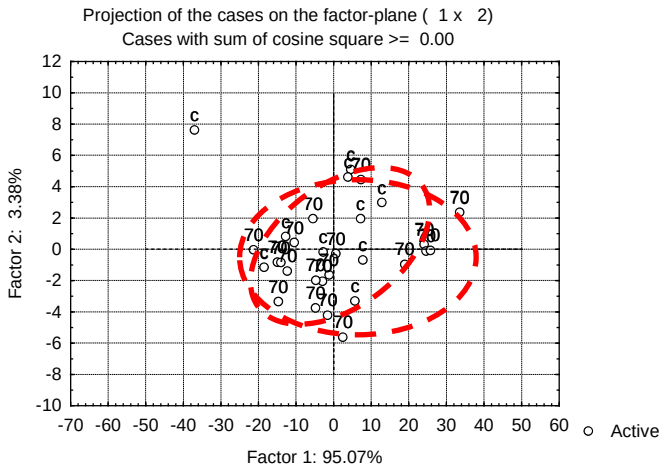
(a). CLA: the unprocessed sample (c; n=10) vs. pellets (p; n=60)



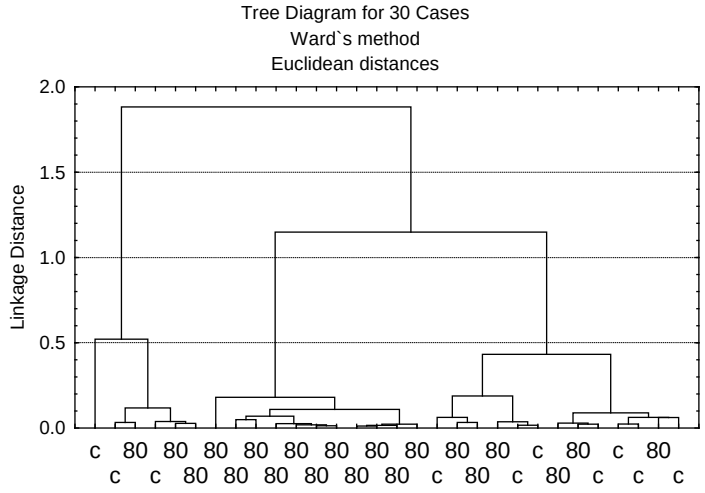
(b). PCA: the unprocessed sample (c; n=10) vs. pellets (p; n=60)



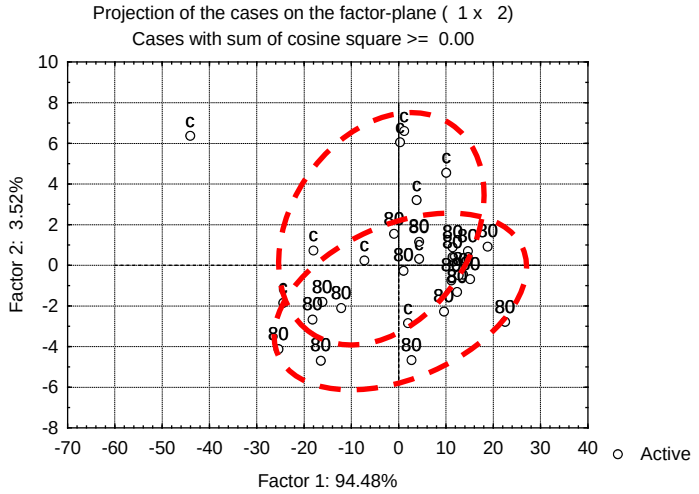
(c). CLA: the unprocessed sample (c; n=10) vs. samples conditioned at 70ºC (70; n=20)



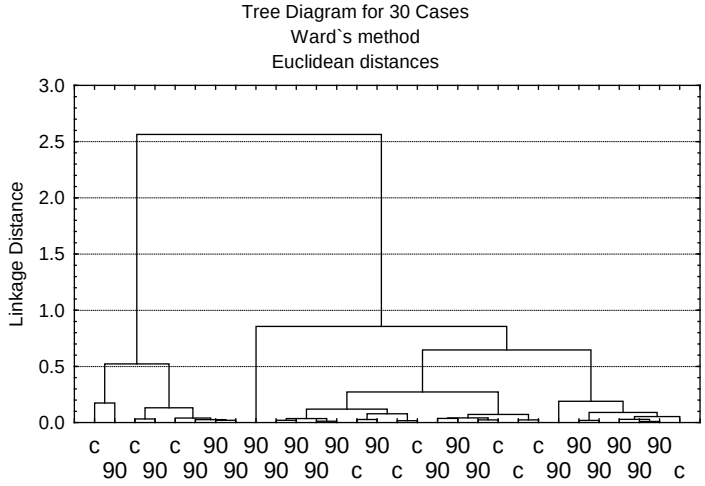
(d). PCA: the unprocessed sample (c; n=10) vs. samples conditioned at 70ºC (70; n=20)



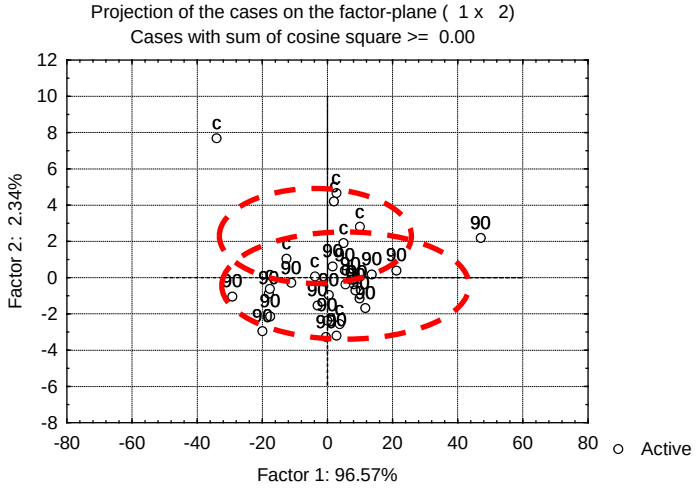
(e). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



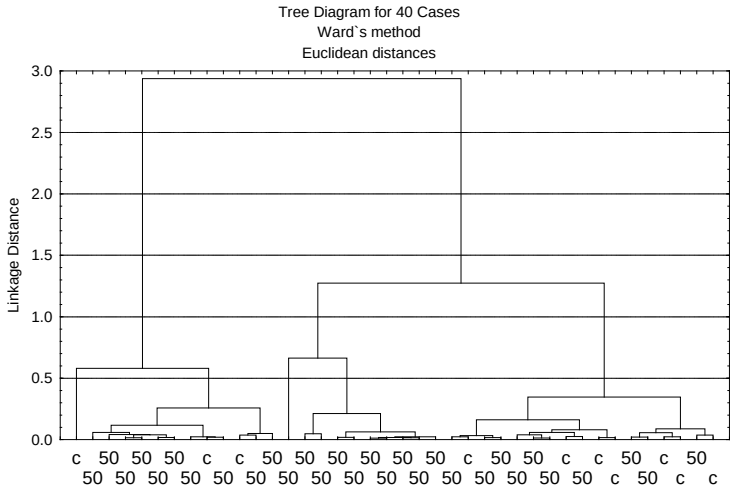
(f). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 80ºC (80; n=20)



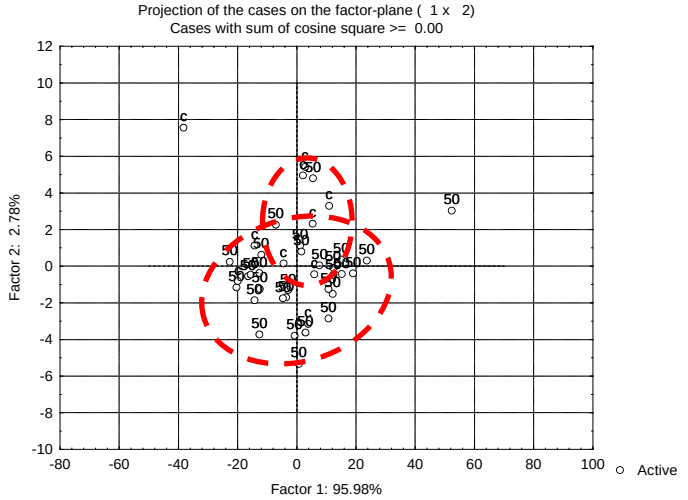
(g). CLA: the unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



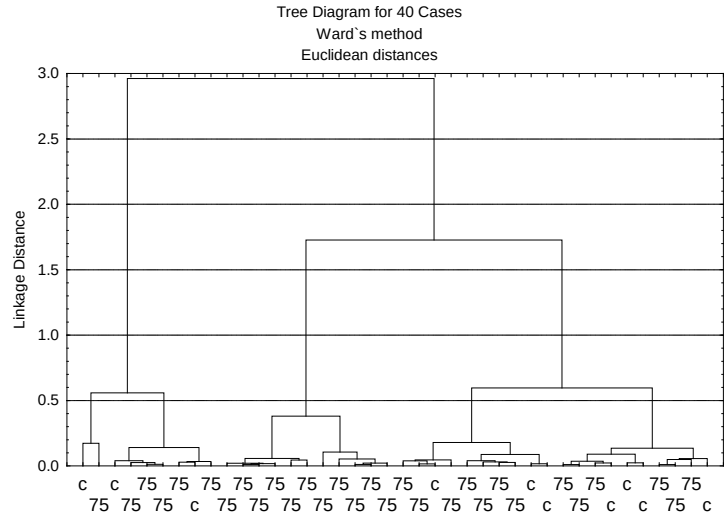
(h). PCA: the unprocessed mash (c; n=10) vs. samples conditioned at 90ºC (90; n=20)



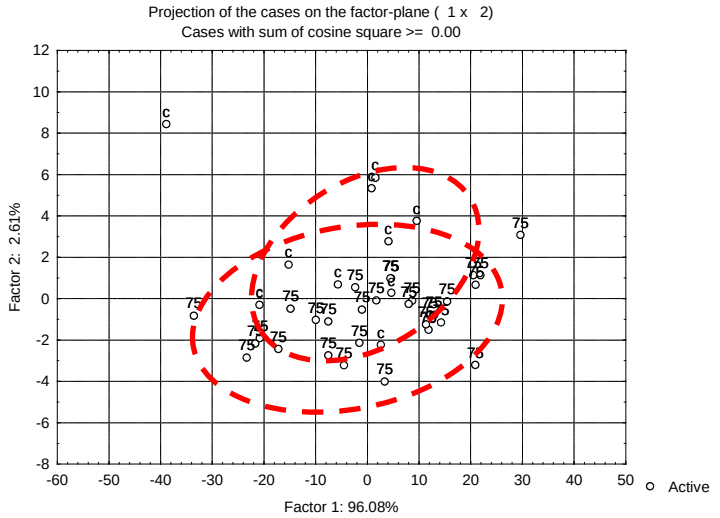
(i). CLA: the unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)



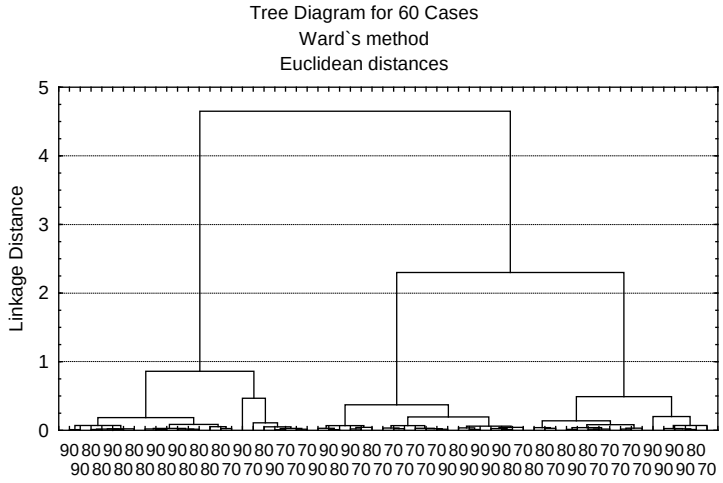
(j). PCA: the unprocessed mash (c; n=10) vs. samples conditioned for 50 sec (50; n=30)



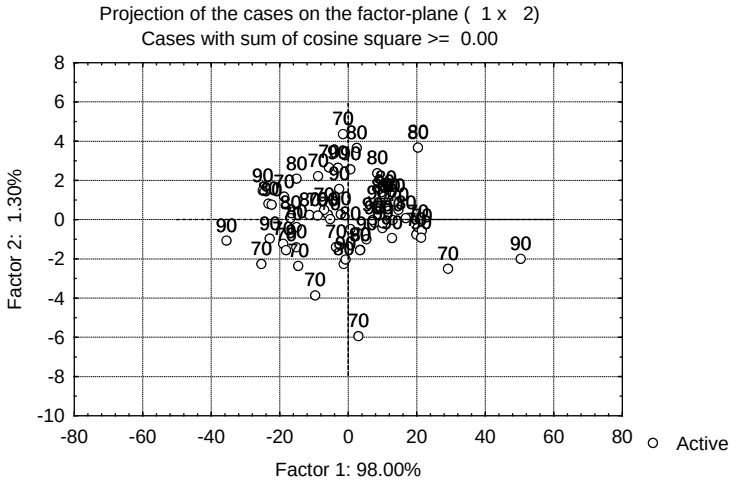
(k). CLA: the unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)



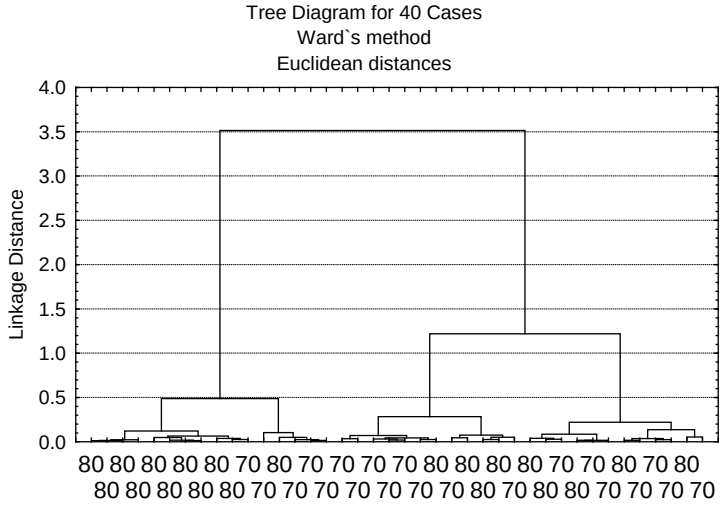
(l). PCA: the unprocessed mash (c; n=10) vs. samples conditioned for 75 sec (75; n=30)



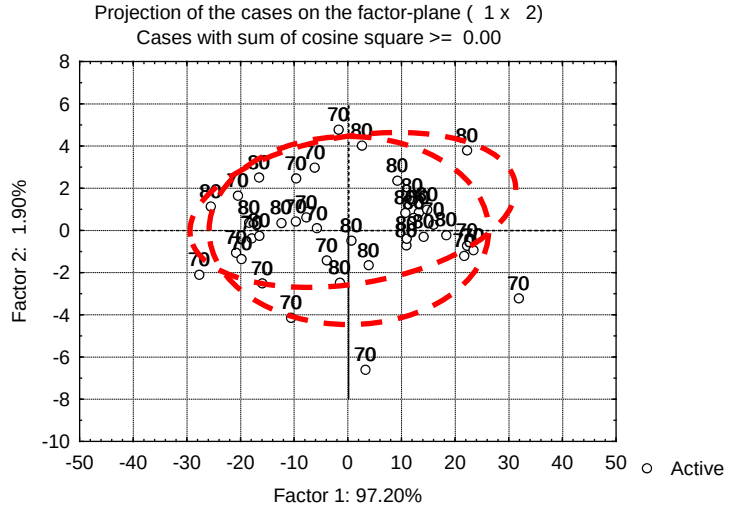
(m). CLA: samples conditioned at 70ºC (70; n=20), 80ºC (80; n=20） and 90ºC (90; n=20)



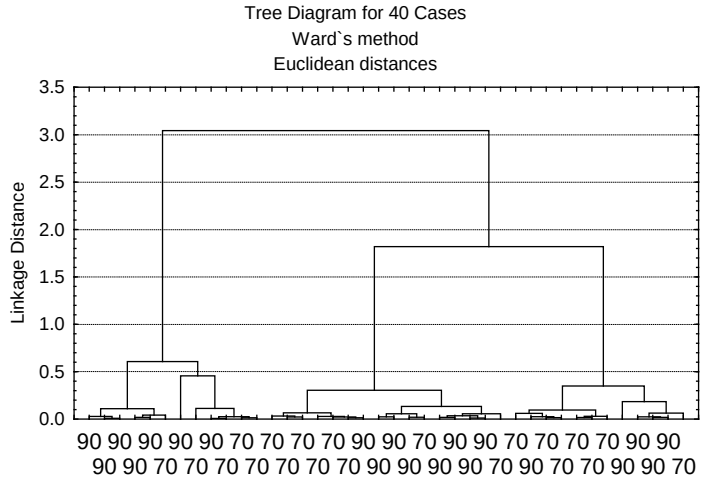
(n). PAC: samples conditioned at 70ºC (70; n=20), 80ºC (80; n=20） and 90ºC (90; n=20)



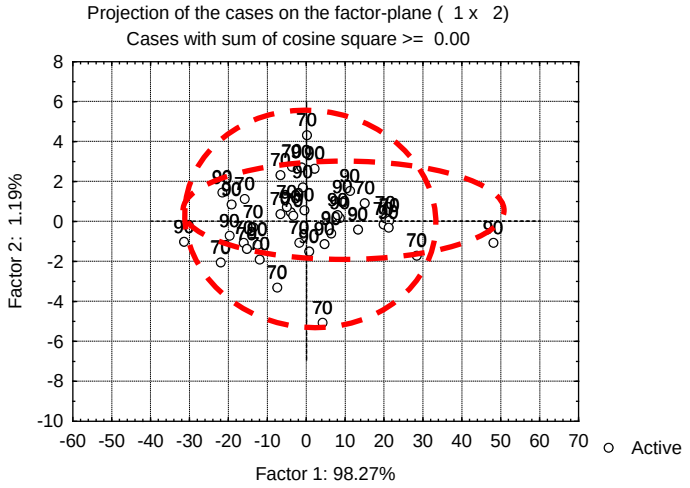
(o). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



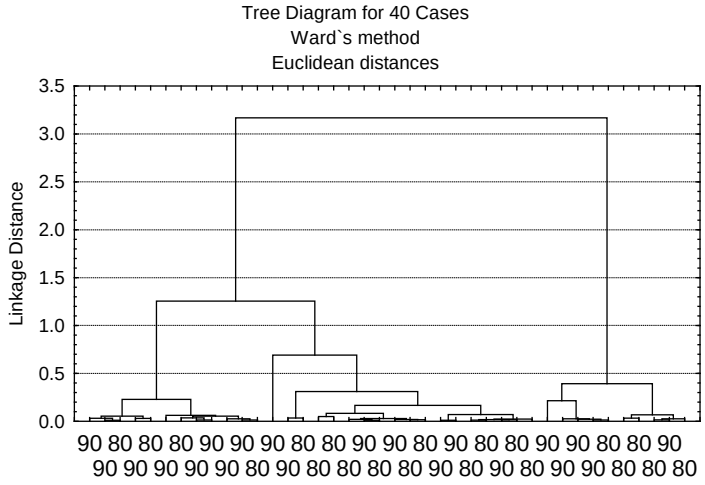
(p). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 80ºC (80; n=20)



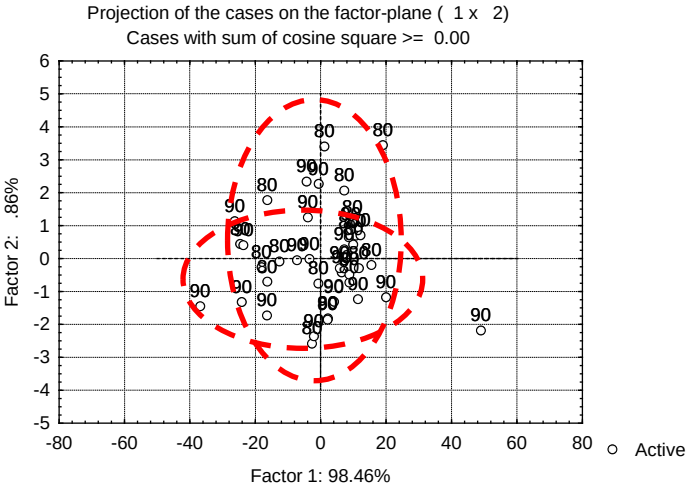
(q). CLA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



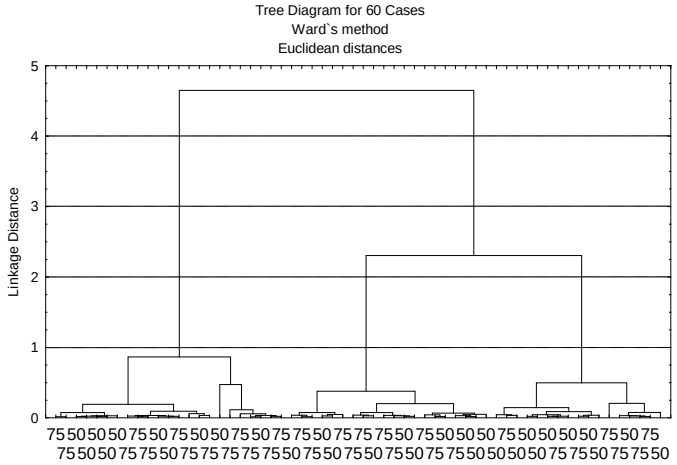
(r). PCA: samples conditioned at 70ºC (70; n=20) vs. samples conditioned at 90ºC (90; n=20)



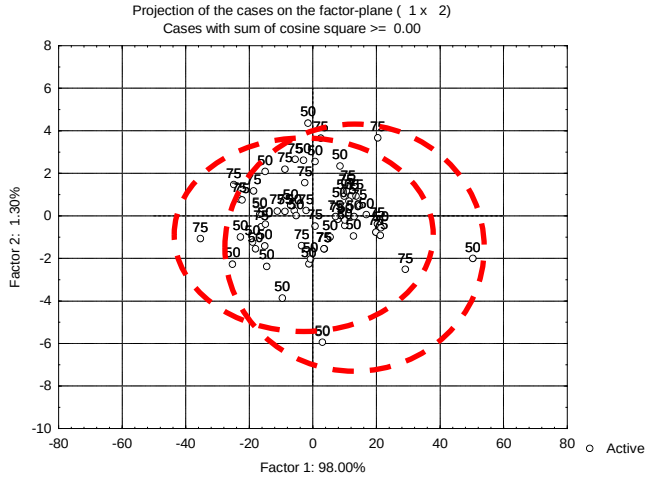
(s). CLA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)



(t). PCA: samples conditioned at 80ºC (80; n=20) vs. samples conditioned at 90ºC (90; n=20)



(u). CLA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec (75; n=30)



(v). PCA: samples conditioned for 50 sec (50; n=30) vs. samples conditioned for 75 sec (75; n=30)

Figure 8.8 Multivariate molecular spectral analyses of TCHO region: ca. 1193-879 cm^{-1}: comparison of samples processed under different conditions. For CLA, Cluster method is Ward's algorithm, and distance method is Euclidean. For PCA, Scatter plots were presented as the 1st principal components (PC1) vs. the 2nd principal components (PC2).